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High-level Meeting on the Establishment of
the International Centre for Genetic Engineering
and Biotechnology

Belgrade, Yugoslavia, 13-17 December 1982

FIVE-YEAR WORK PROGRAMME OF THE INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY*

prepared by

UNIDO Secretariat

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- Selective Application of Advanced Biotechnology for Developing Countries	ID/WG.382/2/Add.1
- Application of Genetic Engineering for Energy and Fertilizer Production from Biomass	ID.WG.382/2/Add.2
- Hydrocarbon Microbiology with Special Reference to Tertiary Oil Recovery from Petroleum Wells	ID/WG.382/2/Add.3
- Application of Genetic Engineering and Biotechnology for the Production of Improved Human and Animal Vaccines with Particular Reference to Tropical Diseases	ID/WG.382/2/Add.4
- Improved Agricultural and Food Products through Genetic Engineering and Biotechnology	ID/WG.382/2/Add.5
- Bio-informatics	ID/WG.382/2/Add.6

INTRODUCTION

1. This note and its addenda present the Work Programme of the proposed International Centre for Genetic Engineering and Biotechnology (ICGEB) for the first five years of its operation. They are intended to serve as the basis for the five-year budget of the Centre and also to provide a general view of the thrust of the Centre's work. It is envisaged that a broad endorsement of the programme would result from the high-level meeting, thereby facilitating the start-up and the operation of the Centre.

2. This note deals with the general aspects of the Work Programme as a whole, while the addenda contain detailed proposals on programme elements. The programme elements and the experts primarily responsible for designing the proposals under them are:

1 Selective Application of Advanced Biotechnology for Developing Countries by Professor Carl-Göran Hedén;

2 Application of Genetic Engineering for Energy and Fertilizer Production from Biomass by Professor Ray Wu;

3 Hydrocarbon Microbiology with Special Reference to Tertiary Oil Recovery from Petroleum Wells by Professor Ananda Chakrabarty;

4 Application of Genetic Engineering and Biotechnology for the Production of Improved Human and Animal Vaccines with Particular Reference to Tropical Diseases by Professor Ahmed Bukhari and Professor Ulf Petterson;

5 Improved Agricultural and Food Products through Genetic Engineering and Biotechnology by Dr. David McConnell; and

6 Bio-informatics by Professor Carl-Göran Hedén.

Each programme element broadly consists of the following: background and justification; activities; work plan; financial requirements; and linkages with other institutions.

3. It is recognized that research and development in genetic engineering and biotechnology is in a particularly dynamic state. Therefore no work programme can be rigidly defined for a period of five years. Besides, the operation of a unique international venture like the ICGEB cannot be spelt out in all detail in advance of its establishment. The Board of Scientific Directors of the Centre 1/ should therefore have an opportunity to look at the programme again and make such elaborations and modifications as may be necessary in the light of the prevailing trends in research and development and other factors.

I. BACKGROUND

4. The idea of an International Centre for Genetic Engineering and Biotechnology was proposed by a number of eminent scientists in February 1981, in a meeting convened by the UNIDO Secretariat for an exchange of views on genetic engineering and biotechnology. 2/ Subsequently, at the request of the UNIDO Secretariat, several experts involved in the meeting participated in a series of missions 3/ to 16 developed and developing countries, during the course of which views were exchanged, inter alia, on the research and development priorities, as perceived by the countries. As a result, while recommending the establishment of the Centre, the report of the experts 4/ also suggested six subject areas to which priority attention may be given in the initial years in regard to research

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1/ See 'The Establishment of an International Centre for Genetic Engineering and Bio-technology (ICGEB), UNIDO/IS.254, page 18, and 'Draft Memorandum of Understanding and Guiding Principles of the International Centre for Genetic Engineering and Biotechnology', ID/WG.382/1.

2/ See Exchange of Views with Experts on the Implications of Advances in Genetic Engineering for Developing Countries, UNIDO/IS.259

3/ The experts who participated in one or more of the programme elements of the missions were: C.G. Hedén, H. Boyer, A. Bukhari, A.Chakrabarty, S. Narang and R. Wu.

4/ See UNIDO/IS.254

and development and pilot plant activities. 5/ The Work Programme present here is in essence an elaboration of proposals in those six areas.

5. Since the publication of the report on the establishment of the Centre, detailed discussions have been entered into with policy-makers and the scientific and technological community in a number of interested countries. Several of them were countries which have not been visited for the purpose of the preparation of the report but were later visited on their request. National-level meetings with policy-makers and the scientific and technological community were also held in some of them. These subsequent exchanges of view have also confirmed broadly the priority areas which had been identified as a result of the first round of field visits.

6. The UNIDO Secretariat requested one expert to take charge of the elaboration of a proposal in each of the six specific subject areas. Before preparing the proposals, the experts met collectively with the UNIDO Secretariat. After the proposals were prepared, the experts met again and reviewed each proposal collectively, paying particular attention to the interlinkages and complementarities among their proposals. An additional proposal on bio-informatics was added, it being considered essential as a service activity for all the others. The proposals under drugs and pharmaceuticals and human and animal vaccines have been merged in one programme element.

7. The Work Programme thus presented in the document is a product of the efforts of a group of high-level experts in this field and is based on the perceptions of the priority needs of developing countries emanating from their discussions in various countries. The UNIDO Secretariat would like to thank the experts for the dedication and high quality of expertise that they have brought to bear on this task.

5/ The six areas were: (i) tertiary oil recovery from petroleum wells; (ii) energy, and fertilizer from biomass, in particular using genetically manipulated bacteria; (iii) improvement of fermentation techniques, particularly of relevance to least developed countries; (iv) development of improved human and animal vaccines; (v) improved agricultural products using phytochrome genes; and (vi) drugs and pharmaceuticals for tropical diseases.

II. CONSIDERATIONS IN THE DESIGN OF THE WORK PROGRAMME

8. The foremost consideration in the preparation of the Work Programme has obviously been that it should accord closely with and promote the objectives of the International Centre. The objective of the Centre, as envisaged in the report on its establishment, is the strengthening of technological capabilities at the national level, particularly in the developing countries; addressing the pressing problems of developing countries, particularly in those areas where concerted R+D attention has not been given; and to bring together for this task the highest level of expertise available in the world. In the words of the expert mission "the mission envisages that the Centre will make fundamental contributions to genetic engineering and biotechnology and will directly help to develop the capabilities of scientists and technologists from the developing countries. These scientists and technologists should then be provided with the necessary means and materials to use their creativity to the benefit of their societies. The primary function of the Centre will be to stress research and development efforts through which scientists and technologists from developing countries will also be trained..... ICGEB must provide facilities that stimulate the cross fertilization between advanced knowledge on the one hand and simple needs and resources on the other and permit the expression of indigenous creativity. Many examples of cross fertilization mentioned in the course of the visits indicated that ICGEB might develop a unique interest profile that could attract scientists from many parts of the world". 6/

9. Having regard to the objectives of the Centre, the experts preparing the Work Programme have taken into account and sought to reconcile several criteria for selection of the elements of the Work Programme, such as:

6/ See UNIDO/IS.254, pages 11 and 8.

(a) the extent of general interest on the part of many countries;

(b) the magnitude of the local need and the potential global impact of the projects;

(c) the technical and scientific feasibility of the projects;

(d) the potential of the projects to trigger off R+D activities and industrial implementation;

(e) the extent to which a contribution could be made to industry as well as other areas, such as food, health, energy and environment; and

(f) the need for a critical mass of efforts for the Centre to produce useful results and to generate worldwide interest and co-operation in this field.

10. In the formulation and review of the proposals, the experts also found that a Work Programme designed to serve the above objectives should balance several considerations, such as the need for selectivity and for advance over a broad front, short-term results and long-term capacities, an effective combination of basic and applied research, and the element of transdisciplinarity which will be a unique feature of the Centre. In addition, they agreed upon the need for providing sufficient flexibility for the implementors of the Work Programme and for effective interaction with ongoing work in the disciplines involved as also the problem addressed. The experts also felt that the proposals should be designed in a modular fashion so as to facilitate implementation.

11. In reviewing the proposals, the experts felt that the Work Programme presented in this document would constitute the minimum needed for a critical mass of effort and for attracting the best talents to be associated with the Centre. It was also considered that the techniques that would be developed through the Work Programme could by no means be considered as too advanced or inappropriate for the developing countries, but may

on the other hand form part of a new type of effort by the international community warranted by the urgency of the problems and the inadequacy of present efforts.

III. THRUST OF THE WORK PROGRAMME

12. The Work Programme, which has been designed keeping in mind the considerations mentioned above, has certain broad features which are discussed below. What is presented is not a summary of the proposals but a brief analytical view of the thrust of the Programme.

13. The question of application of genetic engineering and biotechnology to the present problems of developing countries finds reflection in the programme of advanced biotechnology. The approach, however, is different from the conventional approaches. The aim is to create bio-resources development teams at the national level, and link them to national laboratories, the International Centre providing the role of a concept generator and promoter. Some typical areas in which small-scale pilot plant activities may be carried out are also enumerated in the programme but keeping in view that the activities under other programme elements might provide inputs for small-scale pilot plant activities. Consequently, the role of pilot plant activities in the Centre is considered essential and important. The need for addressing the unique problems of developing countries is reflected in other parts of the Work Programme and applicational aspects are stressed, for example in the development of diagnostic kits for human and animal diseases and the development of enzymes of particular value to food processing. The programme for the tertiary recovery of oil from petroleum wells combines both the basic research for construction of strains and the actual field application of such strains.

14. At the same time, some of the components of the programmes envisage activities starting up from basic research. Apart from the fact that in genetic engineering basic and applied research are closely related, the reason for this can be found in answering the question why the initial thrust of commerical application of genetic engineering has been largely directed towards the biomedical field. 7/ It is because of a large data base provided by many years of basic research in a specialized area of microbiology and the general area of biochemical and biomedical sciences. On the other hand, in several other areas, such as energy, food, crop improvements, biological pesticides, etc., critical basic knowledge has to be acquired in a greater measure. This is also the position in regard to tropical diseases, which have not been the subject of adequate commercial interest on the part of established drugs and pharmaceutical companies. Thus, the programme element on biomass addresses itself to the cloning of cellulase genes, that on fertilizers, to alteration of rhizobia, those on human and animal vaccines and tropical diseases to the molecular biology of the relevant micro-organisms and the programme element on agricultural and food products to the basic understanding of the structure of selected plants. However, the diversity of micro-organisms and plant and animal life warrants a selectivity. The need for addressing the pressing problems of developing countries points to the same need. Consequently, research in regard to cellulase genes will have as a goal the possibility of developing a microorganism which could perform the dual function of converting cellulose to sugar and sugar to ethanol. The research in the field of tropical diseases and human and animal vaccines will have as a goal the treatment of selected human and animal diseases, while the programme relating to food and agricultural products will pay special attention to monocotyledons in order to improve important cereals.

7/ See UNIDO/IS.259, page 9

15. Undoubtedly, links and complementarities exist between the activities, e. g. human and animal vaccines and tropical diseases; and fertilizers from biomass and improvement of agricultural products. The programmes on advanced biotechnology and bioinformatics would provide services "across the board". This mix of activities, in essence, provides a unique combination, not available so far, of transdisciplinarity as well as goal orientation, leading to opportunities for cross fertilization and synergy. 8/

16. The combination mentioned above is particularly necessary, since the Work Programme represents an attempt at capacity building in both applied and basic areas, keeping in view certain broad goals of developing countries. While in certain areas the output of research programmes could be some specific strains or new products or processes, in other areas it might be the building up of basic information and of a capacity for research and application which can be put to use in the respective developing countries on the return of the trainees in a manner suited to their own conditions and responding to their own needs.

IV. WORK PROGRAMME IN PERSPECTIVE

17. The Work Programme proposed has to be seen in the perspective of ongoing activities taking place outside the Centre. The Work Programme itself envisages certain field activities and linkages with institutions. These aspects are dealt with in the next section on the implementation of the Work Programme. Here the broader question of external linkages of the Centre will be discussed. The Council of Scientific Advisers is expected to take into account the external linkages in the implementation of the Work Programme.

8/ See also in this connection the diagram showing the transdisciplinarity in UNIDO/IS.254, page 27.

A. Ongoing Research Work

18. The Work Programme envisages expert groups under each programme element in the initial stages so as to take note of ongoing work, if any, in the same fields. However, a verification has also been made with publicly available information on research projects in genetic engineering and biotechnology.[9/] The verification revealed that only in the case of the programme element on agricultural and food products, some universities in the United States have projects related to the proposals in the programme element. The information collected under the bio-informatics programme will, inter alia, help the Centre to maintain a continuous awareness of ongoing work.

19. In general, it should be remembered that the scope of research in this field is vast and the ongoing work, particularly for the benefit of the developing countries limited. It should also be remembered that the orientation of the research programme of the Centre will be towards developing countries, to suit their conditions and requirements. The same may not hold good in respect of other research programmes.

B. Linkages to International Institutions

20. As the expert report on the establishment of the Centre has emphasized, the Centre is visualized as a unique transdisciplinarity unit. During the preparation of the expert report on the establishment of the Centre and subsequently, contacts have been established with important international bodies, such as United Nations Educational, Scientific and Cultural Organization (UNESCO), World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), International Cell Research Organization (ICRO), European Molecular Biology Organization (EMBO), etc. The establishment of the Centre has been brought to the notice of the Advisory Committee on Co-ordination (ACC) Task Force on Science and Technology for Development and the co-operation of interested organizations has been solicited through Technical Working Group I of the ACC Task Force.[10/]

9/ Genetic Engineering/Biotechnology Sourcebook (McGraw-Hill, Washington, D.C., 1982);

10/ This Working Group is entrusted with the subject of early identification and assessment of new scientific and technological developments and global network of scientific and technological information.

21. The Centre provides an integrated instrumentality for the application of genetic engineering to the solution of development problems. The unique advantage of the Work Programme is that solutions will be attempted not only within a transdisciplinarity framework but also in a milieu where expertise in the field of genetic engineering is pooled and the experience of its application in different areas readily available. The Work Programme, through the confluence of such experience, could assist various development programmes through a new class of technologies not hitherto available. The Work Programme in its relevant aspects is expected to liaise with and promote the aims of different important development programmes, such as those undertaken under the auspices of the tropical diseases programme of WHO and Consultative Group on international agricultural research. After the Centre is established, one of its initial tasks will be to propose and develop specific links with international organizations and their ongoing programmes, keeping in view the requirements of its own Work Programme.

C. National Institutions

22. The strengthening of national institutions being the raison d'être of the Centre, links with them are considered vital for an effective Work Programme. The missions undertaken by the experts and the national meetings held have already provided a broad picture of the types of national institutions, with which the Centre could liaise. The linkages could take various forms, both in the creation of national centres and in supporting them. The Centre's training programme is itself designed to train a core group of scientists who would return to their home countries and start project activities in an institutional framework. Advisory services may be provided for the establishment and operation of national centres. National centres of participating countries, whether already established or to be established, could be regarded as associate centres and complementary R+D work could be carried out in them. The Centre may be able to help to improve the R+D work carried out for the national centres or pass on its work at a defined stage for a national centre to adapt it to local conditions. The

national centres may also help, in due course, in the training function of the International Centre. Co-operation with the national centres will surely be necessary in regard to field work. Also, the formation of Biological Resource Development Teams (BIORED) is a unique innovative step whose success would depend on the co-operation and dynamism of national centres.

23. More specific co-operation with national centres would be sought in a number of ways through each aspect of the Work Programme of the Centre, such as R+D, training, advisory services, etc. It is hoped that the participating countries in the Centre would also pay particular attention to such linkages and come up with appropriate proposals.

D. Linkages with Industry

24. Industrial linkages could be envisaged both up-stream and down-stream. The work done in industry could be carried forward for adaptation, on appropriate arrangements being made with industry. Likewise, the research findings of the Centre, which would require commercialization, would involve linkages with industry. The Council of Scientific Advisers would elaborate appropriate detailed guidelines in this respect.

25. Since external interlinkages will substantially contribute to the effectiveness of the Work Programme and also enhance the Work Programme's effectiveness in an international context, it is envisaged that, as a general rule, before the detailed design and implementation of the programme elements of the Work Programme, an expert group meeting will be held for each programme element, which will, inter alia, examine the possible interlinkages between the programme element and ongoing international and national work.

V. IMPLEMENTATION OF THE WORK PROGRAMME

26. While the details of implementation will have to be left to the management of the Centre, a few general observations would be in order at this stage.

27. In the collective review of the proposals, the experts considered carefully whether or not complete specificity in each case should be provided at this stage. They came to the conclusion that such a specificity would not at this stage be possible in all cases and in some cases may not be desirable. The dynamics of research in this field, the need for flexibility in operation, the capacities and aptitudes of the staff and the trainees and the linkages external to the Centre are factors which militate against a detailed and rigid formulation at this stage. Thus each of the proposals in the addenda should be essentially considered as programme elements for budgetary and programme management.

28. The Council of Scientific Advisers of the Centre will have an important role in guiding the implementation of the Work Programme. This Council will, within the framework of the Programme, consider and approve proposals from the Director of the Centre, spelling out each programme element to the level of specificity needed for implementation. It will in the process take into account the level of scientific and technological effort already reached elsewhere and also the linkages that the particular programme element should develop externally for maximizing its effectiveness.

29. As already stated, the convening of expert groups prior to the implementation of each programme element would provide an opportunity for drawing upon high-level experts, both from the problem area addressed (e.g. industry, energy, etc.) and from the several disciplines which will have a bearing on the research programme. The Council of Scientific Advisers may also, if they deem fit, constitute small project review groups to implement specific programme elements.

30. In view of the transdisciplinarity involved, each programme element may have to be implemented by a team consisting of different specializations. The team may also be drawn from more than one department of the Centre. It is considered that the details of the composition of the research teams and departmentwise implementation should be left to the management of the Centre.

31. In formulating the different programme elements, the experts have felt that the composition of the teams should be somewhat different from what was envisaged in the original report on the establishment of the Centre. The manpower requirements were spelled out in that report in terms of scientists and technologists at fairly senior levels. It is now considered that the ICGEB be staffed with more assistant scientists (equivalent of assistant professors) or technologists and fewer senior scientists (equivalent of full professor or advanced associate professor) or technologists. This modification has been suggested, keeping in mind the modalities of organization of research in different laboratories and talking to a number of scientists in Europe and the United States of America. This would enable wider participation in the Work Programme and in particular benefit younger scientists and technologists. This modification does not, however, affect the budgetary implications already worked out, as will be seen from the chapter on the budget.

32. In regard to the teams themselves, in general, for the implementation of each programme element or its component a senior scientist and technologist must have a support team of at least one junior scientist (assistant professor level), two post-doctoral fellows, and one technician; each senior scientist can train two or three trainees.

33. Each programme element involves a range of activities beginning from training and research to advisory missions, expert group meetings, associates, visiting scientists and post-doctoral fellows, etc. This range of activities will broaden the effectiveness of the programme, both by providing to its inputs of excellence and by creating a larger pool of scientists and technologists who could be associated with and possibly benefit from the implementation. The activities under the programme elements have been phased suitably, bearing in mind that the Centre will reach its full level of operation only in its third year.

34. The Work Programme envisages field activities, wherever appropriate, in order to enhance the suitability of the application of research results. However, clinical trials in the field of drugs and pharmaceuticals is not part of the Work Programme at this stage. As indicated in the respective programme elements, the costs of field activities will in principle be borne by the countries where the activities are undertaken or through other funding programmes. They are not part of the financial requirements of the Work Programme, as indicated in the next section.

35. The experts have felt that in regard to the patenting/licensing of any products, processes and know-how developed in the course of the activities under the Work Programme, the policy should be evolved by the management of the Centre after its establishment. Such a policy should, however, be within the framework of the objectives of the Centre and should be designed to promote those objectives. Within this framework preferential access may be considered for participating countries as distinct from other countries. The experts also generally felt that the key to successful administration of a patent and know-how licensing policy would be flexibility to enable the adoption of the most satisfactory licensing or development option presented by the unique facts of each technological development. It would be premature and unrealistic at this stage to take credit in the Work Programme for any income that may accrue to the Centre, particularly bearing in mind the objectives of the Centre and the general orientation of the Work Programme.

VI. FINANCIAL REQUIREMENTS[11/]

36. The proposals made by the experts for the programme elements yielded a level of financial requirements higher than what was envisaged in the report of the establishment of the Centre.[12/] In their collective review the experts agreed on some reductions taking into account the complementarities of the programme elements and the need to keep as far as possible within the limits of the financial requirements originally envisaged. Accordingly, revised financial requirements have been indicated for each programme element in the respective addenda. They have also been incorporated in the document in the budget of the Centre.[13/]

37. The implementation of the Work Programme is feasible within the framework of the resources envisaged in the report on the establishment of the Centre. Consideration of the effective implementation of the Work Programme have, however, necessitated a few modifications and additions, the more important of which are indicated below.

38. The modifications pertain to:

a) the introduction of a revised concept of research teams[14/] with a number of junior scientists (at lesser salary levels), which permits an increase in research personnel to 50 (10 senior scientist and 40 junior scientists) as against the earlier figure of 30 scientists. The number of technicians has been adjusted accordingly from 30 to 40.

b) the provision for 26 post-doctoral fellows who could be associated in the Work Programme.

These modifications have been made without increasing the personnel costs, envisaged in UNIDO/IS.254, through a revised combination of senior and junior personnel.

11/ See also Proposed Budget of the International Centre for Genetic Engineering and Biotechnology

12/ UNIDO/IS.254, pages 23-24

13/ See also Proposed Budget of the International Centre for Genetic Engineering and Biotechnology

14/ See para 31 ante

39. Additions to financial requirements, in the order of US$ 6 million have, however, been necessitated for the following reasons:

a) The number of man-years for training has been increased from 100 to 200, so as to permit 100 trainees to be trained for two years each;

b) In UNIDO/IS.254, laboratory material costs were indicated for only one year. The need for provision for 5 years for a greater number of research workers has increased the financial requirements under this head. However, only moderate norms, adjusted for different types of personnel (e.g. scientist, junior scientist, technician and trainees) have been adopted so as to economize on materials and to keep the operational costs to the minimum.

40. It should be emphasized, that the foregoing modifications and additions would enlarge the number of scientists and technologists participating in and benefitting from the Work Programme and enable more effective implementation of the Work Programme.

41. The resultant financial requirements for each programme element are indicated below:

	Addendum 1		Addendum 2		Addendum 3		Addendum 4		Addendum 5		Addendum 6		TOTAL	
	Quantity	Thousand US$	Quantity	Thousand US$	Quantity	Thousand US$	Quantity	Thousand US$	Quantity	Thousand US$	Quantity	Thousand US$	Quantity	Thousand US$
Staff Requirements														
Management of the Centre		233.5		233.5		122.5		233.5		142		50		1,015
Scientific and Technological Staff:														
- Senior Scientist	8m/y	600	8m/y	600	8m/y	600	8m/y	600	4m/y	300	4m/y	300	40m/y	3,000
- Junior Scientist	32m/y	1,440	32m/y	1,440	16m/y	720	40m/y	1,800	32m/y	1,440	8m/y	360	160m/y	7,200
- Post-Doctoral Fellow	28m/y	672	28m/y	672	12m/y	288	24m/y	576	12m/y	288	-	-	104m/y	2,496
- Technicians	48m/y	816	32m/y	544	16m/y	272	32m/y	544	24m/y	408	8m/y	136	160m/y	2,720
Supporting Personnel		703.5		703.5		366.5		703.5		428		155		3,060
Subtotal		4,465.0		4,193.0		2,369.0		4,457.0		3,006		1,001		19,491
Visiting Scientists	60m/m	480	40m/m	320	20m/m	160	40m/m	320	40m/m	320	-	-	200m/m	1,600
Expert Group Meeting	4	100	4	100	2	50	4	100	4	100	2	50	20	500
Advisory Services	45m/m	450	30m/m	300	15m/m	150	30m/m	300	30m/m	300	-	-	155m/m	1,500
Training	40m/y	900	50m/y	1,125	30m/y	675	40m/y	900	40m/y	900	-	-	200m/y	4,500
Purchase of Chemicals, etc.		1,620		1,040		560		1,040		760				5,020
Information Material		45		30		15		30		30		600		750
Associateship		150		150		75		150		150		75		750
Miscellaneous (travel, telephone, postage, etc.)		138		138		72		138		84		30		600
		8,348.0		7,396.0		4,126.0		7,435.0		5,650		1,756		34,711

m/y = man year
m/m = man month

Addendum 1 - Selective Application of Advanced Biotechnology for Developing Countries
Addendum 2 - Application of Genetic Engineering for Energy and Fertilizer Production from Biomass
Addendum 3 - Hydrocarbon Microbiology with Special Reference to Tertiary Oil Recovery from Petroleum Wells
Addendum 4 - Application of Genetic Engineering and Biotechnology for the Production of Improved Human and Animal Vaccines with Particular Reference to Tropical Diseases
Addendum 5 - Improved Agricultural and Food Products through Genetic Engineering and Biotechnology
Addendum 6 - Bio-informatics

42. The addenda in the programme elements also contain details of the equipment needed for each of them. Since many items of equipment can be shared for several activities, these requirements would be met from the common equipment, listed in UNIDO/IS.254, page 23.

43. It is, however, important to note that the sum total of the work of the Centre would in a sense be more than that of the sum of its programme elements, both on account of synergy and cross fertilization and also on account of the fact that scientists usually carry on a certain amount of basic research irrespective of the implementation of specific research projects.

VII. ACTION REQUIRED

44. The meeting is requested to consider the Work Programme and, in order that it may constitute the basis for the budget of the Centre and for contributions from participating governments and other sources,

a) agree to generally, endorse the Work Programme for the purposes mentioned above; and

b) recommend it to the ministerial-level meeting for its approval.

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Corrigendum

Page 2, paragraph 3, lines 6-7

For The Board of Scientific Directors of the Centre read The Council of Scientific Advisers

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SELECTIVE APPLICATION OF ADVANCED BIOTECHNOLOGY FOR DEVELOPING COUNTRIES*

prepared by

Carl-Göran Hedén**

* The views expressed in this paper are those of the author and do not necessarily reflect the views of the secretariat of UNIDO. This document has been reproduced without formal editing.

** Professor, Karolinska Institutet, Stockholm, Sweden.

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A. BACKGROUND AND JUSTIFICATION

Modern developments in fermentation technology have deep roots in ancient food technologies that improved the keeping qualities of many perishable products at the same time as they upgraded their taste, digestibility and nutritional value. The early methods for making beer, bread, wine and cheese paved the way for modern bioengineering which now provides the basis for the production of vaccines and other drugs, chemicals, biofertilizers, etc. However, when industrial operations replaced the household practices, pure cultures took over from the natural mixed populations, and the general approach to the practices in fermentation changed. Not only were wooden caskets and concrete or clay vats replaced by stirred stainless-steel reactors, but large-scale production also called for efficient heat exchangers, valves, pipes and many other devices that were not needed for small-scale operations.

The economic consequences of spoiling big batches due to erratic processes also made it necessary to refine the techniques and to set up control laboratories which provided a basis for practically oriented research and development efforts. This tended to emphasize the exploitation of the economy of scale. The optimization of the material inputs and the effective use of instrumentation gradually reduced the labour requirements so that the fermentation industry must now be grouped with "high technology" production processes.

At the same time it must be realized that an internationally competitive production of antibiotics, single-cell protein and many industrial chemicals is now almost unthinkable in anything but very large, modern facilities; it should also be noted that many developing countries have selective advantages that are not fully utilized for lack of appropriate biotechnologies making full use of the recent advances in microbial genetics and bioengineering. Those countries, for instance, often have a great potential for carbohydrate production, cheap labour is available and there is often legislation to guide the industrialization process.

Needless to say, the developing countries will benefit from an indigenous competence making it possible not only to develop methods adapted to their unique opportunities but also to choose equipment and processes from a rapidly expanding international market.

In developing a work plan for the Department for Advanced Biotechnology of the ICGEB there should obviously be a research thrust towards new, simplified biotechnologies - "biodevelopment engineering", for short. However, the department should also have a service function in relation to the other departments and a capacity for training bioengineers so they are well versed in handling advanced equipment for the optimized production of enzymes, drugs, nutrients, etc. Consequently the work plan must be geared to a dual responsibility: research in the field of "biodevelopment engineering" and a service and training function in what might be called "advanced biotechnology".

Almost by definition "biodevelopment engineering" must be field-oriented and transdisciplinary in character. It lends itself to a broadened outlook in fields such as materials science, environmental chemistry and food technology. However, the centre of gravity should be in those areas of applied microbiology which are likely to be of direct relevance to employment generation and rural development. This means that effective methods for small-scale production will be developed and that the technical requirements for the support of such activities will be explored in practice. For instance, the applicability of the "dispersed factory principle" will be studied. Questions would be asked such as: "How can well-controlled starter cultures be produced and distributed to ensure vigorous and safe fermentations in simple equipment?" and "Can immobilized thermophilic strains offer new approaches for village-level fermentations?"

In the selection of research topics suitable for joint efforts at the central facilities of ICGEB, there will certainly be an emphasis on those activities that are not stimulated by conventional "market forces" - either because the potential buyers of a new product are poor, or because a new method would satisfy needs that are only felt in peripheral rural settlements.

ICGEB can also be expected to develop a unique profile in certain segments of the research front. The Centre is for instance likely to strive for excellence in the application of microbial ecology to the design of mixed fermentations that will permit unsterile operations. Such a focus would be more natural at ICGEB than in laboratories where the steam-sterilized stainless steel fermenter is the laboratory centre piece.

The other development objectives of ICGEB, that of providing a service function to the other departments and that of providing a basis for training, must also have a research component, if the goal of fostering internationally competitive bioengineers is to be reached. The emphasis of this component will depend largely on the calibre and specialization of the staff, and the details can consequently not be outlined at this stage. It is, however, obvious that areas such as computer-aided process design, sensor development, energy analysis and mass-transfer theory would all be of relevance.

B. ACTIVITIES

The advanced biotechnology work programme will consist of two distinct types of activities; the field activities carried out through the Biological Resource Development Teams (BIORED) and the research and development programmes carried out at the Centre, including those involving pilot plant studies for the scaling up of processes studied in other work programmes at the Centre.

BIORED

BIOREDs are multidisciplinary teams of local scientists connected to national laboratories of microbiology or the like. Their prior function will be to identify local resources for commercial exploitation and conduct field experiments to explore and develop local biotechnology applications. The role of ICGEB in relation to BIORED will be to promote the development of such teams and provide initial advice and assistance so as to demonstrate the value of this concept for decentralized local applications.

The BIORED will be provided with transportable laboratory equipment for process microbiology, including some basic upstream and downstream equipment for the preparation of raw materials and product recovery in addition to standard fermenters.

A standardized mobile unit would not only serve the extension functions mentioned, but would also permit the testing of novel equipment designed for village use. In addition, it would permit an evaluation of various perishable materials and the suitability of local water resources as a medium base.

This equipment should be designed for flexibility and should be mobile so that it can be assigned to different research and training institutions from which the team could draw laboratory support and facilities for local training courses.

Initially the base laboratories might be chosen among the "Microbiological Resource Centres" (MIRCENs) established by United Nations Environment Programme (UNEP), United Nations Educational, Scientific and Cultural Organization (UNESCO) and International Cell Research Organization (ICRO). They are well co-ordinated and have hosted a number of training courses.

The BIOREDs will be staffed with personnel from the local institute and the operational expenses will be financed by the country hosting the BIORED.

The BIORED missions could well be divided into three four-months periods.

In the first laboratory phase, the unit would work in direct association with a selected base laboratory where local personnel would be trained for field activities.

In the following field phase, the mobile unit would operate in a rural community, or some other suitable target location.

Thirdly, in a technology transfer phase, the unit would assist in the building of suitable facilities and in the starting of processes appropriate to local needs and resources. Some of the core material might well then have to be left behind, but this could be easily be replaced before a new mission is undertaken.

No new mission should start until it is reasonably sure that the base laboratory is capable of providing continued support to the field activities. This technical support ought to be coupled with a socioeconomic impact study. This could provide the basis for social innovations that would help to generate self-reliance (preferential loan systems, marketing contracts, co-operative arrangements, etc.)

In addition to the practical training carried out by the BIOREDs, regional courses will be offered covering a wide range of topics such as fermentation technology, separation processes, etc.

The regional courses would be associated with the initial phase of BIORED operations at a selected base laboratory. This would provide the setting for organized laboratory exercises geared to regional needs, but the practical work would be supported by overview lectures on the state of the art in fermentation technology. The aim of the courses would be to plant the seeds for an indigenous competence ranging from the capacity to evaluate and improve traditional food fermentations to the design of novel equipment based on the experience gained from participation in the BIORED field operations.

Advanced biotechnology and pilot plant

Just as the field activities would be supported by the base laboratory, the latter would be supported by ICGEB. The Centre should not only be capable of trouble-shooting beyong the capacity of the base laboratory, but it would also develop new, simplified biotechnologies, i.e. provide international leadership in "biodevelopment engineering". Since the selection of topics would be guided by the research priorities defined by the scientific board and eventual feedback of BIOREDs, it would be premature to define specific research topics in the current draft work plan. Consequently, the six topics listed below have been selected for their potential to stimulate international co-operation and have fairly general interest:

1. A cheap and simple mycotoxin test for field use. Fungi that produce toxic substances in moist grain, peanuts and other economically important crops are common in the humid tropics, and indigenous fermented foods have also been implicated in occasional episodes of poisoning. Cheap, broad-spectrum detection kits for mycotoxins would therefore be of value for demonstrating the inadequacy of many harvesting, transport, drying and storage procedures. Such kits would also be useful in the search for detoxification methods based on fermentation principles.

2. A microbiological method to release cellulose fibres so that they can be used for small-scale paper manufacturing. It has been demonstrated that mutants of certain fungi that have lost their capacity to decompose cellulose can still attack lignin (7). The process is slow but might be accelerated through the application of genetic engineering and might, under all circumstances, offer a microbiological route to small-scale paper manufacturing where the ambient temperature is high and large containers for wood chips can be built cheaply.

3. An effective system for the production and distribution of mycorrhizae and biological nitrogen fixers that can be used in afforestation. It has been demonstrated that afforestation of arid environments can be supported by inoculation with mycorrhizae and that nitrogen-fixing microorganisms can also be put to use in forestry. However, mycorrhiza spores are difficult to produce in significant quantities, and reliable and cheap carrier materials for the distribution of various inoculants are needed.

4. A protected fermentation to propagate B. thuringiensis under primitive conditions, for use as an insecticide. Flies constitute a health hazard in many rural communities and, even if emphasis must be given to the hygienic upgrading of the environment, a biological insecticide of proven efficacy and safety might be a useful adjunct.

5. A fermentation method to preserve and upgrade leaf protein so that it can be used as a baby food. It is now well known that the leaf protein, which can be pressed out of many plants, is often of excellent nutritive qualitiy. However, it is a perishable material that might need to be preserved. An interesting approach is then to use fermentation with lactobacilli (8), a process which might become part of systems designed to utilize the biomass from every part of the plant. Almost all components of such systems (effective lactic acid production, bioconversion of lignocellulosic residues, fermentative upgrading of the juices left after protein removal) could benefit from genetic engineering.

6. Thermophilic cellulase producer(s) for small-scale use in the production of liquid fuels. Thermophilic cellulase producers are of particular interest because they can be used in "unsterile" fermentations that are suitable for warm climates where they require little or no water cooling. If, in addition, they themselves, or in conjunction with other

thermophilic microorganisms, can convert the glucose produced into alcohol (or other volatiles) a subsequent distillation process for liquid fuels would require less energy.

This activity is considered complementary to those under the work programme on the "Application of Genetic Engineering for Energy and Fertilizer Production from Biomass".[1/]

Research targets such as those mentioned above would pave the way for co-operation with the other research projects. Advanced labelling techniques and monoclonal antibody techniques would for instance be natural components of item 1, genetic engineering of 2 and 6, and microbial physiology of 3,4 and 5.

These research targets listed here might also trigger international co-operation beyond ICGEB and its associated institutions. An exchange of strains with the MIRCEN laboratories (1) might for instance contribute significantly to success with project 3, and international screening for thermophilic cellulase producers could be an important component of project 6.

However, as mentioned earlier, the advanced biotechnology programme would also provide a service function to the other work programmes which would often require quantities of specialized enzymes, antigens, and other fine chemicals that would call for large-scale production runs. Those would be performed according to advanced bioengineering principles and would thus introduce visiting scientists to a "competitive fermentation technology" that makes full use of modern optimization techniques and computer aids. A likely focus would be on such restriction enzymes and advanced reagents as a young scientist might need after returning to his home country.

The Centre needs facilities for large-scale work and supporting work shop facilities in view of two critically important functions:

(a) To provide adequate quantities of important organic materials to the various departments and other research laboratories engaged in genetic engineering and biotechnology but lacking facilities for preparatory work. This service would be provided on a pure cost basis (man hours, chemicals and basic services such as steam and electricity) but without regard to the capital investment in the equipment;

1/See ID/WG.382/2/Add.2

(b) To provide for scale-up experiments on novel products and processes and to give a setting for training in modern bioengineering practices (fed-batch fermentations, feedback control based on head-space analysis and calorimetry, data logging and computer-assisted optimization, etc.)

Considering the variety of projects that might require support, the pilot plant would have to be designed for a very high degree of flexibility. It would be split into three separate sections. One would maintain the heavy equipment (air compressors, steam generator, auxiliary power supply and refrigeration system) along with three fermenters (100, 1000 and 5000 litres) of the standard common in the antibiotics industry.

The second section would be a safety facility with air locks, exhaust gas filtration, effluent and overflow sterilization, steam seals or magnetic transmissions for rotating shafts, special alarms for malfunctions, etc. This facility would also have three big fermenters (100, 500 and 1000 litres). In addition it would have a closed system for harvesting and cell disruption, so that enzymes and antigens could be extracted from pathogens.

The main function would, however, be to perform scaling-up experiments on genetically manipulated microorganisms.

The third section would service the two others with a combined gas chromatograph-mass spectrometer, oxygen and carbon dioxide analyzers for the control of pH, temperature, aeration, stirrer speed, etc. in the various fermenters, all of which could be put under computer control.

In addition to the experience and know-how obtained by participating in the above-mentioned activities, the trainees will complete their education through formal courses.

The central courses would be aimed at methods suitable for the preparation of various important enzymes and other biochemical materials used in genetic engineering and applications of enzyme technology. (A more detailed description of the formal education in biochemical engineering is provided in Annex II) The exercises would be based on a laboratory manual that would be subject to continuous upgrading on the basis of the feedback from participants in earlier sessions and on new data that is published.

The laboratory manual would be divided into four parts:

A. laboratory hygiene and safety;
B. strain maintenance and control;
C. production techniques; and
D. separation and purification methods.

Its preparation and updating would be the responsibility of a permanent joint committee with participation from all departments. The result should be a useful manual for in-house use but also a loose-leaf handbook that would help to keep all associated laboratories and the ICGEB alumni up to date with regard to modern preparatory methods.

Once a year, the work programme would organize a small meeting for a project review group of experts. This meeting would cover important technical developments relevant to the activities of the department. Participating governments would be invited to send observers to this meeting, which should be designed as a seminar that would provide an overview of the fermentation field, including important patents and licensing agreements. The papers presented at the seminar and a summary of the discussions would be distributed to ICGEB alumni and participating governments as a series entitled "Bioengineering for Development".

C. WORK PLAN

Year 1:

- Planning of facilities and establishment of research priorities by the Board of Scientific Directors in consultation with a project review group;
- Design of pilot plant;
- Design of model BIORED unit (No. 1).

Year 2:

- Testing of pilot plant equipment;
- Establishment and assignment of BIORED Unit 1;
- Negotiations about BIORED Units 2 and 3;
- Preparation of laboratory manual;
- First central course.

Year 3:

- BIORED Unit 1 operates in location A;
- BIORED Units 2 and 3 built and assigned;
- First regional course at location A;
- First expert meeting and seminar;
- Second central course.

Year 4:

- BIORED Unit 1 operates in location B;
- BIORED Units 2 and 3 operate in locations C and D;
- Negotiations about BIORED Units 4 and 5;
- Regional courses at locations B,C, and D;
- Third central course.

Year 5:

- BIORED Unit 1 operates in location E;
- BIORED Units 2 and 3 operate in locations F and G;
- BIORED Units 4 and 5 built and assigned to locations H and I;
- Regional courses at locations E,F.G,H and I;
- Second expert meeting and seminar;
- Fourth central course;
- Evaluation performed by project review group.

D. PREREQUISITES

- Government interested in forming BIORED Teams;
- National Laboratories willing to support BIORED;
- The qualified personnel needed to constitute BIORED Teams.

E. FINANCIAL REQUIREMENTS

The staff required to successfully carry out the work programme outlined above will consist of:

- two senior scientists or technologists;
- eight junior scientist or technologists;
- seven post-doctoral fellows;
- twelve technicians.

It is expected that in the five years twenty trainees will receive formal training in biotechnology.

The Centre will provide the equipment necessary to carry out the activities of this work programme. The required equipment is in Annex I.

Five-year Budget

STAFF		(US$ thousands)
(first year 40 per cent, second year 60 per cent of full operation)		
Senior scientist	8 man year	600
Junior scientist	32 man year	1,440
Post-doctoral scientist	28 man year	672
Technicians	48 many year	816
Subtotal		3,528
Management of the Centre and Supporting Personnel		937
Total Staff		4,465
OPERATIONAL ACTIVITIES		
Visiting scientists	68 man months	480
Expert group meetings	4	100
Advisory services	45 man months	450
Training	40 man years	900
Information material		45
Purchase of Chemicals, etc.	108 man unit years	1,620
Associateship		150
Miscellaneous (travel, telephone, etc.)		138
Total Operational Activities		3,883
Total Work Programme		8,348

ANNEX I

EQUIPMENT REQUIREMENTS

A. BIORED

BIORED use will be:

1. Self-contained in the sense that it should satisfy its own needs for electricity, steam, compressed air and refrigeration (diesel generator and electrical compressor, oil-heated boiler and heat exchanger for evaporative cooling);

2. Capable of grinding, sieving, extracting, boiling and filtering raw materials and of separating microbial cells from liquid media. This equipment would be selected with special reference to the possibility of translating the results obtained into equipment that is appropriate for local use (bamboo tubes, containers of sisal cement or ferrocement, filters of coconut husk, etc.). Centrifugal separation would only be used on a small scale for demonstration and training purposes;

3. Easily transportable: This would limit the size of the fermenters so that they would yield useful results without becoming cumbersome (3 x 10 and 1 x 150 litres stirred reactors and on 75-litre tower fermenter designed both for liquid cultures and for use as an immobilized cell column). In addition there would be one 100-litre media preparation vessel which could also be used for anaerobic cultures or as an incubator for semisolid fermentations.

The equipment mentioned (except the three small fermenters) would be designed to be carried on a open trailer that could be pulled by a four-wheel drive diesel truck. This would carry the service equipment mentioned under 1 above. The 10-litre fermenters would be housed in an air-conditioned trailer laboratory that would also contain an incubator, a refrigerator and a temperature-controlled shaker table. The instrumentation needed for running all the BIORED equipment would be located in the trailer, which would also accommodate two bunks for the support staff. The laboratory trailer would be provided with telecommunications equipment, as would also its pulling vehicle, a large diesel jeep. In addition, this would satisfy the general transportation needs.

Besides supporting research and training activities aimed at such things as the production of seed inoculants, dairy starters, etc., in liquid culture, the BIOREDs should also be equipped with easily transportable materials such as bag digestors for biogas production. In addition, the equipment should include design drawings and audiovisual aids as well as tools and materials for building various devices like plastic-lined ponds (for producing clean water), reflectors (for solar sterilization of media and for the drying of materials) as well as different types of enclosures for semisolid fermentations, mushroom cultivation, etc.

A wide range of processes that deserve attention have been outlined by C. Seshadri and his group at Madras (3) with reference to applied photosynthesis, and by K. Steinkraus (4) in relation to food fermentations. In addition, the review "Microbial Processes: Promising Technologies for Developing Countries", published by the United States Academy of Sciences (5), and the newsletters distributed by the International Mushroom Society for the Tropics (6) deserve careful study in the course of planning individual BIORED missions.

B. EQUIPMENT FOR ADVANCED BIOTECHNOLOGY AND PILOT PLANT

Equipment

- Standard laboratory equipment (microscopes, incubators, refigerators, centrifuges, gas chromatographs, fraction collectors, spectrophotometers, etc.);
- Ten fully instrumented small-scale (10-15 litre) fermenters, mobile, for use outside the biotechnology department;
- Stock of industrial type glassware.

Pilot Plant

- Carbon ring and standard compressors;
- Steam generator;
- Fermenters, including filters, valves and other fixtures:
  one 100-litre unit,
  one 1000-lite unit,
  one 5000-litre unit;
- Storage and precipitation vessels;
- Continuous media sterilizer;
- Harvesting equipment;
- Drying equipment;
- Pipes, platforms, telfers, etc.

Safety Section

- Special safety features (ventilation, sewage, etc.):
- Fermenters, including filters, valves and other fixtures:
  one 100-litre unit,
  one 500 -litre unit,
  one 1000-litre unit;
- Equipment for harvesting and cell disruption;
- Equipment for product recovery and purification.

Control Section

- Gas chromatograph/mass spectrometer;
- Three mobile O_2 and CO_2 units;
- Other standard instruments;
- Standard instrumentation (calorimetry, fluormetry, nephelometry, autoanalysers, etc.);
- Computer.

ANNEX II

FORMAL EDUCATION IN BIOCHEMICAL ENGINEERING

(a) Aims

The ICGEB should produce two types of expert bioengineers: genetic engineering and biochemical engineers. Needless to say, their training would partly overlap, so that a developing country that would have to build bioengineering "from scratch" could initiate an infrastructure around either of the two types of expert.

In the following section only the training of biochemical engineers will be considered. This is done against the background of IUPAC's 1974 "Recommendations on Education in Biochemical Engineering" (12). This presupposes a four-year academic training which should begin with fundamental studies at the undergraduate level, and it accommodates students oriented both towards biology and towards engineering.

The ICGEB training should be broad-based and structured in such a fashion that the knowledge gained does not easily become obsolete. The biochemical engineers produced would have to be "jacks of all trades" with a capacity to control, innovate and trouble-shoot in the fermentation industry and to act as consultants on matters as diverse as management of water pollution, design of fermention plants and elimination of hazards in biochemical work. Of course, they might also be called upon to fill university posts, where they would be charged both with education and research.

(b) Background of Students

Obviously the training must be more flexible and individualized than that we see in industrialized countries, where there are many employment opportunities that require specialization in mechanical, chemical, biochemical, food and environmental engineering, etc. For setting the wheels in motion towards such a differentiation, where biochemical engineering is a component, a developing country

might start with undergraduate courses in food technology and then in fermentation engineering and waste/pollution control. This might be followed by an expansion of the same fields with post-graduate courses, including areas such as enzyme engineering and biomedical engineering. The way would thus be paved towards an indigenous academic and industrial research capability, but since this is inadequate in most poor countries they are more likely to use returning ICGEB trainees as crystallization nuclei for further development.

An adequate training in bioengineering requires knowledge from three basic disciplines: chemical engineering, biochemistry and microbiology. However, without a foundation in reaction kinetics involving microorganisms, biochemical energetics and microbial conversion processes, the translation of various mathematical representations into strategies for controlling biosystems is extremely difficult. Consequently, most graduates from developing countries need a supplementary training where their basic analytical skills are developed and where they learn to think in terms of systems and unit processes.

The focus of their basic training might vary, but in most cases chemical engineering would have been the starting point for a supplementary education in biochemistry and microbiology and for gaining some industrial experience. Most students would consequently come from engineering colleges where undergraduate courses of a year or so would have provided the basic knowledge of general chemistry, physics and mathematics. The graduate training might have started with a year of physical chemistry, organic chemistry, chemical engineering, statistics and computer science supplemented with some basic courses in microbiology. In a third and fourth year there might then have been a focus on practically significant studies of heat transfer, mechanical operations, kinetics and instrumentation and on a minor, experimentally oriented, individual project.

Ideally, the students would come from universities where biochemical engineering was emerging through courses in food technology covering thermal death and chemical activities of microorganisms as an introduction to an overview of the fermentation industry.

However, ICGEB should also be prepared to accept students who had gone through college with an orientation towards medicine and the life sciences, provided and they had supplemented their physics and chemistry with a solid base in mathematics.

In summary then, acceptance for ICGEB postgraduate training would presuppose a four-year academic education starting with fundamental subjects at an undergraduate level followed by differentiated studies oriented either towards engineering or towards the life sciences.

(c) ICGEB Postgraduate Programme in Biochemical Engineering

Students with an adequate background in microbiology, genetics, biochemistry, organic chemistry and chemical engineering might be considered for a 1-2 year postgraduate fellowship programme at institutions associated with ICGEB. The training of the student would then be planned and a thesis topic selected in a dialogue with ICGEB and an associate institution. Both basic and applied projects would be selected (1:1). The associated institution would assume the responsibility for granting degrees.

Since the training would be individualized there is no point in presenting a curriculum, but reference should be made to the courses already offered at the Biological Research Centre of the Hungarian Academy of Sciences (Szeged) and at the International Centre of Co-operative Research and Development in Microbial Engineering, Japan (Osaka). Also, note should be taken of the experience gained in the early 1970s from the "Brazil Chemistry Program". This was an experiment in postgraduate research and training that is highly relevant to the ICGEB's impact aims.

There is for instance every reason to emphasize that ".... overseas Research Fellows need special help in locating new positions when they return home. Provision should be made for travel grants and other devices after the programme is finished to make possible ongoing scientific collaboration among scientists of the participating countries" (13).

The observation that "developing countries with strong capability in a particular discipline might usefully form a linkage with another developing country that happens to have relatively less strength in the chosen field" should also be noted in the planning of individual training programmes.

A start would be made with advanced training in the disciplines that provide the scientific basis of fermentation technology (biochemistry, microbial physiology and genetics). This would be followed with a certain specialization depending on the student's aptitude and ought to include some industrial experience. There would either be an industrial orientation, with an emphasis on process design and economics, or the aim would be set for a regular academic career. Both lines would qualify for advanced doctoral training at ICGEB.

(d) Research Training at ICGEB

The doctoral and post-doctoral training at ICGEB should be informal and based on internal seminars and on lectures and demonstrations by invited specialists. The student should finish the thesis that he had initiated at the associated institution that would eventually grant him a degree. He should gain broad laboratory experience through an individual programme that would rotate him through all ICGEB departments over a period of a few years. The operation of fermenters, biochemical reactors and product recovery equipment would be emphasized, and periods of work in the support (media kitchen) and maintenance facilities (workshop) would be obligatory.

The training would also include exposure to mission-oriented case studies selected to illustrate those problems of design and process engineering that a bioengineer would face when called upon to initiate, plan and implement projects in developing countries. Emphasis would for instance be given to problems related to the utilization of data banks and other sources of information, to the management of technology transfer and licensing, and to the principles that govern the policy with regard to microbiological patents in various countries.

(e) The Alumni Programme at ICGEB

Every effort should be made to maintain the links between ICGEB and the experts which the Centre had trained and between the various "growing points" which the alumni may have established in developing countries. At their disposal the Board of Scientific Directors should consequently have a fund from which, once a year, they could distribute travel grants for this purpose to the alumni.

REFERENCES

1. "MIRCEN NEWS", regular Newsletter from UNEP/UNESCO/ICRO, c/o Dr. T. Rosswall, Dept. of Microbiology, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden.

2. Draft Medium-Term Plan (1084-1989), First Part, General Conference of UNESCO. Fourth Extraordinary Session. Paris, 1982. 4xc/4.

3. Newsletters from Shri A.M.M. Muragappa Chettiar Research Center (Photosynthesis and Energy Division), Tharmani, Madras 600042, India.

4. Steinkraus, K.H., "Application of Biotechnology and Genetic Engineering to African Fermented Food Processes". Paper presented at Abijan, Ivory Coast, April 5-9, 1982.

5. "Microbial Processes, Promising Technologies for Developing Countries". Report by an ad hoc panel to the United States National Academy of Science, Washington, D.C., 1979.

6. Newsletter from the International Mushroom Society for the Tropics, c/o Dept. of Biology, The Chinese University of Hong Kong. Shotin, N.T. Hong Kong.

7. Erikson, K.E. and Goodell, E.W.: Can.J. Microbiol. 20. 371. 1974

8. Szakács, G.Y. and E. Madas. "Wet preservation of leaf protein by lactobacillus. Biotech. and Bioeng. 21.721. 1979.

9. Hedén, C.-G. "The Potential Impact of Microbiology on Developing Countries". UNIDO/IS.261, Nov. 27th, 1981.

10. Hedén, C.-G. "Global Aspects of Biotechnology." J.Chem. Tech. Biotech. 32, 18, 1982.

11. "Computer-based conferencing systems for developing countries". IDRC, Oct. 26-30,1981.

12. "Recommendations on Education and Biochemical Engineering". Fermentation Committee of the International Union of Pure and Applied Chemistry (IUPAC Information Bulletin, No. 48, p.68, 1974).

13. "The Brazil Chemistry Programme - an International Experiement in Science Education". The Commission on International Relations of the Board on Science and Technology for International Development of the U.S. National Research Council, National Academy of Science, Washington, D.C. 1979.

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APPLICATION OF GENETIC ENGINEERING FOR ENERGY AND FERTILIZER PRODUCTION FROM BIOMASS*

prepared by

Ray Wu**

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** Professor; Department of Biochemistry, Cornell University, Wing Hall, Ithaca N.Y. 14853, USA.

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A(1). BACKGROUND AND JUSTIFICATION

In the light of the growing problems of fuel shortages and waste recycling it is necessary to establish efficient use and conversion of renewable resources (such as cellulosic materials and wastes) to liquid fuels. This will replace, at least in part, the need for the dwindling supplies of non-renewable materials such as petroleum oil.

In order to solve this important world-wide problem, input from genetic engineering and biotechnology is absolutely essential. Genetic engineering represents a multiple array of disciplines, which includes biochemistry, microbiology, genetics and recombinant DNA technology.

1. Cellulosic materials. Cellulose and hemicellulose are the most abundant renewable resources in nature. With lignin, they form about 95 per cent of the dry weight of plant wastes. World production of waste cellulose exceeds four billion tons per year (1). If cellulose materials can be efficiently converted into ethanol, a tremendous source for liquid fuel will be made available. Technically speaking, the process is relatively simple - the hydrolysis of cellulose to glucose and subsequent conversion to ethanol. The process was used for large scale production of ethanol during the First World War and the Second World War, and was further refined in the 1940s. Due to the low yield and corrosion by the catalytic acid needed to break down cellulosic materials, the process was not economically competitive with the petroleum-based processes. However, the recent energy crisis and the rapid exhaustion of the petroleum reserves, together with the development of microbial enzymatic hydrolysis of cellulose, has gradually reversed this situation. Through the implementation of genetic engineering and biotechnology, it is hopeful that the conversion of cellulosic material to ethanol will become economically competitive.

2. Cellulases. The major cellulase components were isolated from culture filtrates of many microorganisms such as Trichoderma reesei, the most thoroughly studied system. By using chromatographic procedures, the cellulases have been purified to homogeneity (2). These include:

(a) 1,4-β-glucano glucanohydrolase (also known as endoglucanase, CMCase - converts amorphous cellulose to free fibres.

(b) 1,4-β-glucan cellobiohydrolase (also known as exoglucomase, exocellulase, avicellase) - converts filter paper or avicel to cellobiose with terminal mode of attack. It is a decrystallizing enzyme. Cellobiose is a competitive inhibitor.

(c) β-glucosidase (also known as cellobiase) - converts cellobiose (also possible cellotriose and cellotetrose) to glucose. Glucose acts as a noncompetitive inhibitor. Apparent high substrate inhibition at very high substrate concentration. It is the least stable of the three cellulases, and is present in the least amount in T. reesei.

3. Cellulolytic microorganisms. Many microorganisms contain cellulases. A few that have received recent attention are as follows (3):

(a) Bacteria
Cellvibro fulvus, cellvibro vulgaris - aerobe, mesophile;
Cellulomonas - aerobe, mesophile;
Pseudomonas fluorescens - aerobe, mesophile;
Clostridium thermocellulaseum - anaerobe, thermophile

(b) Actinomycete (aerobic)
Streptomyces QMB814
Thermoactinomycete; thermomonospora curvata;
Thermomonospora fusca

(c) Fungi (aerobic)
Aspergillus niger
Trametes sanguinea; Poria; Pestalotiopsis westerdijkii
Penicillium iriensis QM9624; Penicillium funiculosum;
Polyporus versicola; Polyporum tulipiferae;
Fusarium solani; sporotrichum pulverulentum QM9145
Trichoderma reesei (viride)
Trichoderma lignorium; Tricoderma koniugii
Thermoascus aurantiacus QM9383

Since the biomass is comprised of about 45 per cent cellulose, about 30 per cent hemicellulose and about 20 per cent lignin, the central theme and major objective of this work programme is the efficient conversion of cellulose and hemicellulose to ethanol. There are several experimental approaches to accomplish the above objectives. Only two of the most important ones will be discussed here:

(a) Using genetically manipulated microorganisms;

(b) Using recombinant DNA technology especially for molecular cloning and amplification of cellulase genes.

B(1). ACTIVITIES

For the effective conversion of cellulose and hemicellulose to ethanol, a number of fundamental as well as technical problems have to be solved. It is clear that several different problems will have to be addressed in parallel. Much of this work programme will involve basic research since a number of fundamental points in this complex system remain to be established.

1. Analysis of Cellulases from Cellulolytic Organisms. The three cellulases, the endoglucanase, the exoglucanase and the cellobiase, from a variety of known cellulolytic organisms (see section A(1)) will be analyzed for the amount excreted into the extracellular medium and in the cells for the β-glucosidase (e.g. units/ml), the specific activity of crude and purified enzymes (units/mg protein), the stability, the extent of product or substrate inhibition, etc. Other microorganisms should be screened for new sources of cellulases.

Once several suitable sources of cellulases are found, the amount or the properties of the enzymes can most likely be improved by applying genetic methods of mutation such as those used to select the favourable Trichoderma reesei mutants AM9414, MCG77 and MCG80 (4).

2. Cloning of the Cellulase Genes and Producing Favourable Mutants by in vitro mutagenesis. One suitable research approach that ICGEB can take is to clone the cellulase genes from cellulolytic organisms analysed in section one above. This can be accomplished by either making genomic libraries of these organisms or by making cDNAs from the mRNAs by applying the recombinant DNA techniques (5,6). Once the genes are cloned in a plasmid, placed next to a strong promoter and suitable ribosome binding sites, and introduced into E. coli or B. subtilis by transformation procedures (5,6), production of large amounts of these cellulases are expected.

The next step is to carry out in vitro mutagenesis on the cloned cellulase genes by base pair changes or by small deletions (7) with the hope of producing mutant genes which code for cellulases with increased resistance to product inhibition.

3. Cloning of fungal Cellulase Genes and Introducing them into Yeast. The fungus, Trichoderma reesei, produces and excretes large amounts of cellulases which can degrade cellulose to glucose. Yeast cells (such as Saccharomyces cerevisiae) that can efficiently convert glucose to ethanol cannot degrade cellulose. One suitable research approach that ICGEB may consider is to construct an improved strain of yeast which carries the cellulase genes and can excrete cellulases out of the cells to convert cellulose to glucose. This novel strain should be able to efficiently convert cellulose to ethanol. The construction of the strain involves two steps. The first step is to clone the genes for the cellulases of Trichoderma reesei (mutant MCG77 or MCG80) or of other cellulolytic fungi as described in the previous section. However, in this case the genes are joined to a yeast plasmid (such as YEp13 type plasmid shich has an Ars sequence and an easy selectable marker such as His3 or LEU2). The second step is to transfer the cellulase genes together with the yeast plasmids into yeast by transformation (8). The plasmid can be maintained in the yeast by growing the yeast in the absence of leucine. Another possibility is to allow the cellulase genes to be stably integrated into the yeast chromosome. Assuming that Trichoderma reesei signal peptides are recognized by the yeast (quite likely since both T. reesei and yeast are fungi) the cloned cellulase genes should include signal peptides for excretion of the cellulases into the medium.

If they are not recognized by the yeast, an alternative approach can be taken. The cloned gene coding for the T. reesei signal peptide will be removed by standard recombinant DNA techniques and only the structural genes of the cellulases will be included. These cellulase genes will be joined to a derivative of the YEp13 which also contains the promoter sequence and the signal peptide sequence of the yeast invertase gene. Since the invertase of yeast is excreted, the cellulase genes joined to its signal sequence gene should also be excreted. Yeast mutants which over-produce invertase have been isolated (9). If necessary, the invertase gene from this strain of yeast will be cloned.

The advantage of this approach is that common yeast cells such as S. cerevisiae (for which the transformation system has been established, and which can easily be grown in large scale fermenters) can then convert cellulosic materials directly to alcohol.

4. Cloning of Bacterial Cellulase Genes and Introducing them into Zymomonas mobilis. The bacterium Zymomonas mobilis can convert glucose to ethanol (10) with the following advantages:

(a) high specific ethanol yields;

(b) a tolerance to high glucose concentrations; and

(c) the ability to grow totally anaerobically so that the addition of oxygen in continuous fermentations is unnecessary.

Recently, Bland et al. (12) used an "attached film expanded bed" (AFEB) cell-immobilization reactor (13) and showed that the conversion of sugar to ethanol with the bacterium Z. mobilis can be increased to 105 gm/l-hr based on total fermenter volume (12). Although the AFEB fermenter is an efficient way to convert sugar to ethanol by Z. mobilis, this bacterium does not have the cellulase genes to convert cellulose to glucose.

Bacteria like Cellulomonas fimi contain cellulase genes and secrets cellulases that penetrate the wood matrix and attack the cellulose extracellularly to produce sugars. At least one cellulase from C. fimi has been cloned recently (11). The next step is to clone the gene coding for all three cellulases (endo-β 1,4-glucanase, exo-β 1,4-glucanase,

β-glucosidase) together with the signal peptide coding sequence, and join them to a bacterial plasmid such as pKC30 (14) next to the strong promoter sequence as described in section two above.

One project ICGEB can pursue is to transfer the cloned bacterial cellulase genes on a plasmid such as the pKC30 into Zymomonas mobilis by transformation. It may be necessary to first work out the transformation procedures for introducing plasmids into Z. mobilis by employing the principles and techniques used in introducing plasmids and foreign genes into E. coli and B. subtilis. If the plasmid and the cellulase genes are not stable in the bacterium, they can be forced to integrate into the bacterial chromosome by integrative recombination. This is accomplished by first cloning a gene Y from Z. mobilis in the same plasmid pKC30. Once the cellulase genes are also joined to this plasmid and transform the cells, the entire pKC30 derivative can be integrated into the chromosome of Z. mobilis at the site of gene Y by integrative recombination.

5. Cloning of xylose isomerase Gene and Introducing it into S. pombe.
High concentrations of hemicellulose-derived carbohydrates can be easily obtained by the use of dilute acids. The ideal organisms for the conversion of biomass-derived carbohydrates to products such as ethanol are the yeasts. However, most yeasts are not able to degrade pentoses. The recent successful demonstration of a quantitative production of ethanol (80 per cent of the theoretical yield) from D-xylose using yeasts and xylose isomerase would simplify the problems related to the pentose process (16).

One possible line of research is to clone the xylose isomerase gene and introduce it into a Schizosaccharomyces pombe, which is a more desirable yeast than baker's yeast (S. cerevisiae) in the conversion of xylulose to ethanol. This genetically manipulated yeast can then convert xylose to ethanol at high yield. This yeast strain should be much more efficient in ethanol production than the recently discovered strain of Pachysolen tannophilus strain NRRL 2460 (17).

In addition to the production of ethanol, the byproducts of hemicellulose degradation by microorganisms include methane, organic

acids, sugar alcohols, solvents and animal feed. Thus, other valuable materials can be produced.

In fact, this part of the work programme emphsizes the use of genetically manipulated yeasts or bacteria for the efficient conversion of cellulosic materials to ethanol. It is clear that the same yeasts or bacteria would also be useful in converting biomass into feed (such as single cell protein), and industrial chemicals as mentioned in the above paragraph.

The major component of the programme would be the research and development component, where research work would be conducted by at least four closely inter-related groups. The first to be led by a microbiologist, whose major function will be to analyze the cellulases from the known cellulolytic microorganisms and to look for new cellulolytic microorganisms. The second group, to be led by a biochemist, will function to purify the cellulases (selected by the microbiology group) and to study their properties. This group will also initiate work on the cloning of the cellulase genes. The third group, to be led by a recombinant DNA expert, will function to modify the cloned cellulase genes (by the biochemistry group), and also to transfer the cloned bacterial cellulase genes in Zymomonas mobilis cells. The fourth group, to be led by a expert in yeast, and will function to transfer the cloned Tricoderma reesei cellulase genes (or other fungal cellulase genes such as those from Penicillium funiculosum) into yeast cells, and to establish conditions for the optimal conversion of cellulose to glucose and to ethanol. Each of these four groups will include a postdoctoral fellow and/or a technician, and the group leader of these groups will be assisted by a junior scientist (Ph.D. level). Each group can train about two trainees from developing countries every two years.

Two meetings or workshops will be conducted by these four groups. International experts from each area of research and development will be invited. The topics of discussion will include studies on cellulolytic microorganisms and cellulases, the cloning and amplification of the cellulase genes, the transfer of cloned cellulase genes into fermentative yeast or bacteria, the improvements of the efficiency of conversion of cellulose (and hemicellulose) to sugar and then to alcohol.

The outputs of this programme will be produced in several steps. The first step will aim to identify the best cellulases (from one or more cellulolytic microorganisms), which give stable and high specific activity cellulases (the endoglucanase and the exoglucanase should be excreted into the growth medium). The next step is to clone several of these cellulases and join them to bacterial plasmids or yeast plasmids. The third step is to transfer the cloned fungal cellulases (such as those from T. reesei) into yeast cells and to maximize the direct conversion of cellulose to ethanol in this recombinant yeast. Parallel experiments will be carried out to transfer cloned bacterial cellulases (such as those from Cellulomonas fimi) into Zymomonas mobilis so that this recombinant bacteria can convert cellulose directly to ethanol. Substantial progress is expected; however, completion of research and development within the next five years would be unrealistic. However, even a partial sucess will be significant in ultimately solving the problem of efficiently and economically converting cellulosic materials to ethanol. Once basic R+D has been completed, the programme will be handed to the biotechnology group to adapt to large scale production of ethanol. Considerable technological improvements in the large scale recovery and drying of ethanol is also needed.

Advisory services will be provided to developing countries laboratories in these areas.

PRODUCTION OF FERTILIZER USING BIOLOGICAL SYSTEMS

A(2). BACKGROUND AND JUSTIFICATION

Biological nitrogen fixation is an essential process for all living organisms on the earth. The ability to reduce atmospheric nitrogen to ammonia is limited to a small number of bacteria, blue-green algae and actinomycetes. Some of these microorganisms can reduce atmospheric nitrogen in free-living form such as Klebsiella, Azotobacter, and blue-green algae. The economically important microorganisms, however, carry out the fixation process in close association with higher plants in the form of true symbiosis. During symbiosis the higher plant contributes the essential carbohydrates to the microorganism, and in turn the microorganisms supply the reduced nitrogen for the bacteria and higher plant protein synthesis. The nitrogen fixation, an anaerobic process, is carried out by the nitrogenase enzyme complex which is highly conserved in all nitrogen-fixing microorganisms. The entire process in Klebsiella pneumoniae is encoded by a gene cluster containing 17 contiguous genes (three structural genes) which are transcribed unidirectionally in seven transcriptional units. N_2 fixation Nif operon requires the constant presence of the gene A protein which is functioning as an inducer. The gene L protein acts as a repressor of the Nif operon by regulating the production of gene A protein. Recent data of several laboratories demonstrate, not only in Klebsiella but also in Rhizobia and blue-green algae, that the Nif genes are organized in contiguous clusters. The regulation of Rhizobia nitrogen fixation process is presently not understood.

Symbiotic nitrogen fixation requires the interaction of microorganisms with higher plant cells. The proliferation of the plant root, induced by Rhizobia, results in nodule formation in leguminous plants. In contract to crown gall formation, the Rhizobia are inside of the plant cells and in a developmentally regulated process become bacteroids which carry out the fixation process.

Plant recognition, the infection process, and the host specificity seem to be carried as gene clusters in the bacterial genome. Recent findings in Rhizobium leguminosarum document the presence of nodulation genes and nitrogen fixation genes on a large plasmid. When this plasmid was transferred to Agrobacterium tumifaciens, ineffective non-fixing nodules were formed after infection of pea roots. Similarly, Rhizobium meliloti cells contain even larger plasmids called megaplasmids (larger than 500 KD in size). These megaplasmids also contain genes required for the nodulation process closely linked to the nitrogen-fixing gene cluster. Experiments to transfer the nitrogen-fixing capability from bacterial cells into a eukaryotic cell system by genetic engineering resulted in stable maintenance of the Nif gene cluster in yeast chromosome number 3. However, no translation products were observed in the transformed yeast cells, suggesting that the replacement of the bacterial Nif gene signals by yeast or plant signals is required to obtain gene expression. Further, more detailed studies will be required to understand the assembly of nitrogenase in a eukariotic cell.

B(2). ACTIVITIES

For the production of fertilizer using biological systems, a number of fundamental as well as technical problems have to be solved. Only two important projects are presented here. The research work would be conducted by a team of at least three closely inter-related groups, and in consultation with the plant genetic engineers as described in the programme of Improved Agricultural and Food Products.[1/] The first group, to be lead by a microbiologist, will work with nitrogen-fixing bacteria such as Rhizobium, Klebsella and Azotobacter, and to study the efficiency and host range for nitrogen fixation. The second group, to be led by a biochemist, will use biochemical methods to analyze the nitrogen fixation system, and use recombinant DNA technology to clone and to transfer genes between nitrogen-fixing strains of bacteria or between bacteria and plants. The third group, to be led by an expert in plants and plant cells, will study the mechanism and efficiency of nitrogen-fixation and photosynthetic systems in plants.

1/ "Improved Agricultural and Food Products through Genetic Engineering and Biotechnology", ID/WG.382/2/Add.5

As the systems becomes better understood, he will co-operate with the microbiology and biochemistry groups to use the genetically selected nitrogen-fixing bacteria or to transfer recombinant DNA modified bacteria into plants. He will check the rate of plant-growth, the protein content in seeds, etc., and try to maximize the yield.

Each of these three groups will include a postdoctoral fellow and a technician, and perhaps a junior scientist who will assist the group leader for this programme. Each group can train about two trainees from developing countries every two years. In other words, the three groups can train six trainees in two years, or 15 trainees in the first five years.

A joint annual meeting or workshop will be conducted by these three groups. Experts in this area of research from different countries will be invited to participate.

Advisory services from several major research groups in the world in this area of research will be needed. The advisory services will include inviting active researchers to come to the ICGEB for several days to several weeks. Perhaps three to six researchers will be invited each year. The services may also involve sending one or several researchers to certain major research centres around the world to work out very specific problems, related to soil conditions and competitive strains of nitrogen fixing bacteria in that region. Those strains may be brought back to the ICGEB for genetic modification.

The major output of this programme will be to improve the existing strains of Rhizobium to make them more competitive in their natural environment in different areas of the world. Since the soil conditions and the competing bacteria in the soils in different parts of the world will be different, tailor-made improved strains of Rhizobium will be made in each case. Although the principle of genetic modification is quite simple, this area of research is time-consuming and major break-throughs within the first five years may not be possible. On the other hand, even minor improvements in nitrogen-fixation efficiency, which leads to a ten per cent increase in crop yield, will be economically significant.

Furthermore, any basic research at the ICGEB which leads to a better understanding of the nitrogen-fixing bacteria, and on the bacteria-plant interaction will be beneficial in improving crop productivity. Soybean and cowpeas are used as examples in this work programme. The principle behind this type of research and the technology gained can be applied to other systems.

The use of free-living microorganisms for improving crop productivity by studying nitrogen-fixing Pseudomonas and blue-green algae, is likely to yield positive results during the first five years.

1. Use of Genetic Engineering to Alter Thixobium and Other Mircroorgans to Fix More Nitrogen for Improved Crop Production

Alteration of Rhizobia for Improved Crop Productivity.

The major limiting factors in symbiotic nitrogen fixation are the lack of competitive strains of Rhizobium. It is well known that less than five per cent of the inoculated Rhizobia are found to be infective on growing crop plants. Secondly, Rhizobia are needed which are capable of nodulating and fixing nitrogen in the presence of fixed bacteria in the soil. Thirdly, the nodulation process and the nitrogen fixation process are sensitive to stress factors, e.g., drought, salt, and heat.

The major leguminous crop plants are soybean and cowpeas. Therefore, emphasis should be put on isolation of novel strains from soils and selection of competitive strains of Rhizobium japonicum and Rhizobium cowpea species. At the same time, these strains should be adopted to regional plant cultivars and to local soil conditions. Recombinant DNA technology, upon the availability of Rhizobium gene transfer systems, could be used to combine beneficial traits of natural Rhizobia. In addition to the generation of competitive strains, application of molecular biology to Rhizobia could lead to constitutive nodulator and constitutive fixer strains which are independent or which cannot be inhibited by soil nitrogen. Upon the availability of gene transfer systems and cloned genes in slow-growing Rhizobia nodulation genes, nitrogen fixation genes or the ability to overcome stress could be mobilized to create superior Rhizobium strains.

Efforts to understand response of the plant upon infection with microbes should also be approached by molecular biology. The ability of microorganisms to overcome natural plant defense mechanisms can lead to more effective Rhizobia infection and nodulation. Efforts also should be made to create nodules on other portions of the plant other than the root, for example, the stem or leaves.

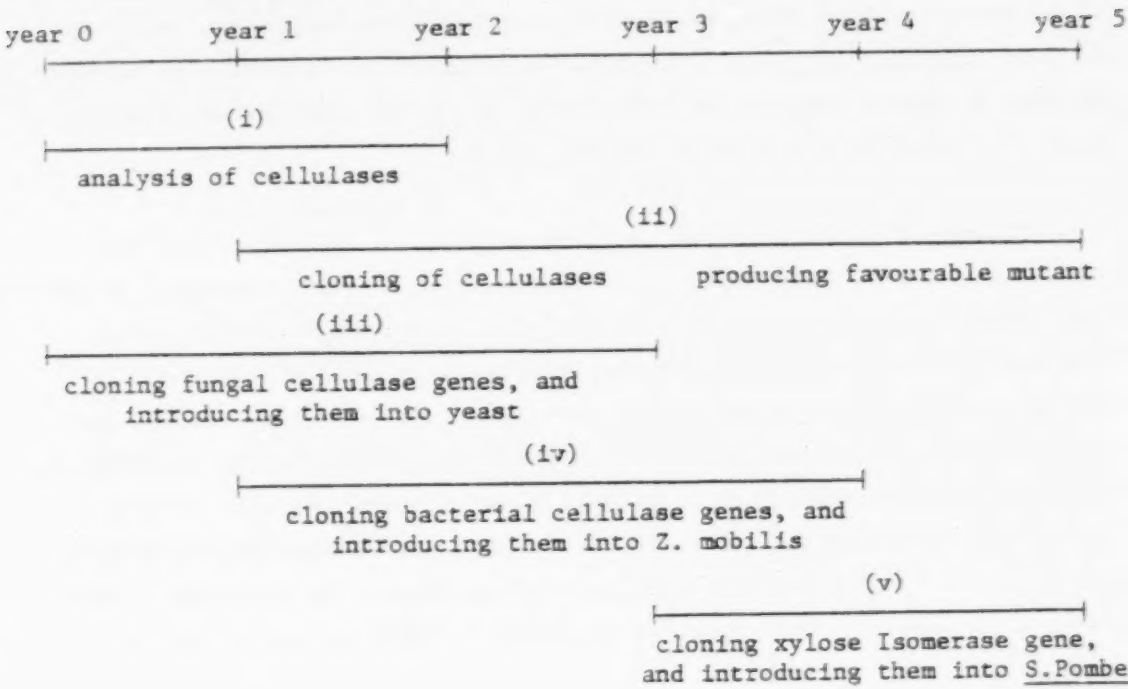
The supply of nitrogen in the form of nodules on stems or leaves, at the most needed time before pod filling, could be of tremendous economical importance in agriculture. Furthermore, the interaction between photosynthesis and nitrogen fixation should also be studied at the level of molecular biology. In summary, the development of superior strains, the more complete understanding of the infection process, and the response of the higher plant could lead to optimal plant-microbe association and, therefore, increased productivity in legumes. Recombinant DNA technology may allow us to transfer the nitrogen-fixing ability to important plants other than legumes. This research is strictly basic in nature and will take a long time to realize.

2. Use of Free-Living Microorganisms for Improving Crop Productivity.

Major nonleguminous crop plants are corn, rice, wheat, etc. The replacement of chemically reduced nitrogen by biologically fixed nitrogen in these crops is of immense economical importance; therefore, soil microorganisms, such as Azotobacter, nitrogen-fixing Pseudomonas and blue-green algae should receive more attention. The possible application of root or stem associated nitrogen-fixing Pseudomonas might become as important for fixation in cereal grains as Rhizobia are for increased legume production. In addition, genetic engineering on soil microorganisms in general could lead to better crop production by engineering microorganisms incapable of causing diseases or by altering microorganisms to become natural antagonists of pathogens and competitors. Further, attention should be directed to photosynthetic microorganisms which could be adapted as symbionts on leaves of higher plants; one such example is the waterfern (Azolla/blue-green algal interaction.

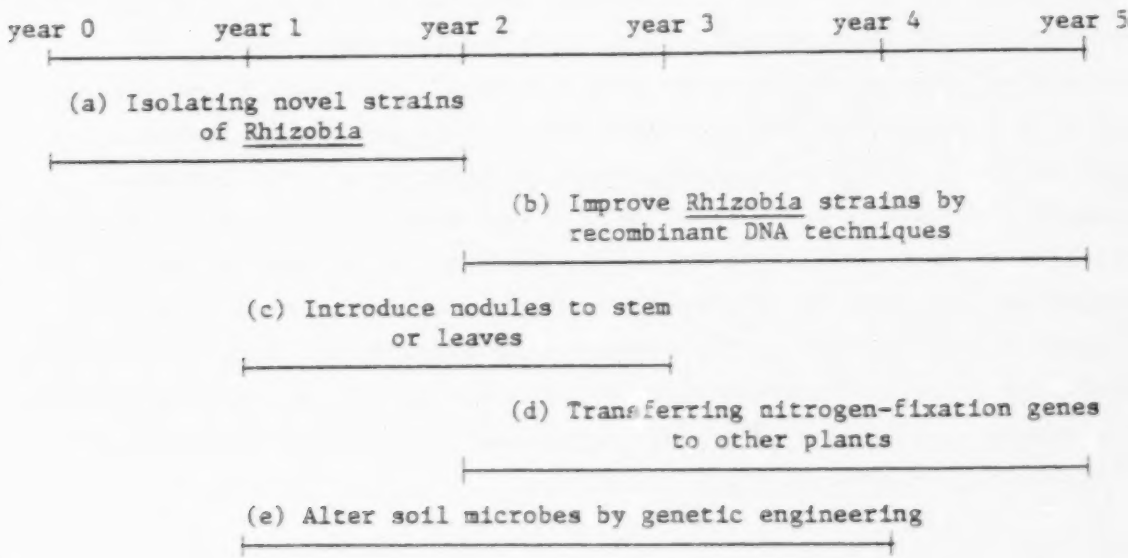
C(2). WORK PLAN

Time table for the work programme on fuel production from biomass:



Time table for the work programme fertilizers by biological system:

Some projects are expected to give positive results within the time frame indicated, others (such as (b) and (d)) may need much longer time for completion.


year 0
year 1
year 2
year 3
year 4
year 5
(a) Isolating novel strains of Rhizobia
(b) Improve Rhizobia strains by recombinant DNA techniques
(c) Introduce nodules to stem or leaves
(d) Transferring nitrogen-fixation genes to other plants
(e) Alter soil microbes by genetic engineering

D(2). COLLABORATION WITH OTHER INSTITUTIONS

Contacts with several major research institutions will be needed. Those will include:

- Advanced research laboratories;
- relevant international organizations like the Food and Agriculture Organization of the United Nations (FAO);
- ongoing projects in this area, e.g. UNIDO programme on Conversion of Agricultural Wastes in the Philippines.

E(2). PREREQUISITES

The prerequisite necessary for ensuring the successful implementation of the programme is the appropriate academic qualifications of the trainees. The trainees must have an equivalent of a Ph.D. degree in biochemistry or microbiology. They must be familiar with the general laboratory techniques in biochemistry and microbiology. They must be highly motivated and eager to learn under close supervision from the scientific staff members or postdoctoral fellows at the ICGEB.

Initially, candidates for trainees may be recommended by a scientific board in a developing country. It is imperative that the final selection be made by the scientific staff at the ICGEB, because qualifications must be based on scientific merit.

F(2). FINANCIAL REQUIREMENTS

The following staff will be required when the programme will be in full operation:

- two senior scientists
- eight junior scientists
- seven postdoctoral fellows
- eight technicians

25 trainees are expected to be enrolled in this work programme.

Five-Year Budget

		(US$ thousands)
STAFF		
(first year 40 per cent, second year 60 per cent)		
Senior scientist	8 man year	600
Junior scientist	32 man year	1,440
Post-doctoral scientist	28 man year	672
Technicians	32 man year	544
Subtotal		3,256
Management of the Centre and Supporting Personnel		937
Total Staff		4,193
OPERATIONAL ACTIVITIES		
Visiting scientists	40 man months	320
Expert group meetings	4 man months	100
Advisory services	30 man months	300
Training	50 man year	1,125
Information material		30
Purchase of chemicals, etc.	104 man unit year	1,040
Associateship		150
Miscellaneous (travel, telephone, etc.)		138
Total Operational Activities		3,203
Total Work Programme		7,396

ANNEX I

EQUIPMENT REQUIREMENTS

The equipment requirement for this programme is as follows:

- 30 water baths and incubators
- 20 small rotary shakers
- 20 balances (one micro, one macro)
- 10 pH meter
- 20 desk top microcentrifuges
- 20 refrigerated microcentrifuges
- 10 floor model medium speed centrifuge (such as Sorvall RC-5)
- 10 spectrophotometer
- 30 refrigerators

The shared support facilities will include:

(a) Instrument rooms with:

- 1 scintillation counter
- 1 ice machine
- 2 large rotary shakers
- 1 recording spectrophotometer
- 10 electrophoresis sets
- 2 floor model medium speed centrifuges
- 2 high speed centrifuges (such as Beckman L8-70)

(b) A biological containment room (p2 level) with:

- 2 laminar flow hoods
- 2 large rotary shakers
- 2 medium speed centrifuges
- 1 high speed centrifuge
- 1 autoclave
- 2 microscopes
- 2 water baths
- 2 incubators

(c) A media preparation and sterilization room with:

- 1 autoclave
- 1 sterile hood
- 1 glass-distilled water still
- 2 balances

(d) A 4° cold room with:

- 1 small centrifuge
- 2 water baths
- several columns for chromatography

(e) A -20° cold room.

(f) A dark room with:

- 1 automatic x-ray film developer
- 1 enlarger
- 1 printer

(g) A dishwashing room with:

- 2 dishwashing machines
- 1 autoclave
- 2 ovens, etc.

Temperature and humidity controlled group chambers or greenhouses will be needed to grow plant cells and plants.

REFERENCE FOR THE LIQUID FUEL FROM BIOMASS

1. A.E. Humphrey (1979) in Hydrolysis of Cellulose. Mechanisms of Enzymatic and Acid Catalysis (Eds. R.D. Brown, Jr. and L. Jurasek) p. 25, Amerc. Chem. Society, Washington, D.C.

2. Gong, C.S., Ladisch, M.R. and Tsao, G.T. (1979) in Hydrolisis of Cellulose: Mechanism and enzymatic and acid catalysis (Eds. R.D. Brown and L. Jurasek), 261.

3. Mandels, M. and Andreotti, R.E. (May, 1978) Process Biochem., pp 6-13.

4. Andreotti, R.E., Medeiros, J.E., Roche, C.R. and Mandels, M. (1981) Proc. Second Int'l. Sympo. on Bioconversion, New Delhi, India (Ed. T.K. Ghose), Vol. 1, 353-372.

5. "Methods in Enzymology" (1979) Recombinant DNA (Ed. R. Wu) Vol. 68.

6. "A Manual for Genetic Engineering: Advanced Bacterial Genetics" (1980) (Eds. R.W. Davis, D. Bostein, J.R. Roth), Cold Spring Harbor Lab.

7. Smith, M. and Gillam, S. (1981) Genetic Engineering (Eds. J.K. Setlow, A. Hollaender) 3, 1.

8. Hinnen, A., Hicks, J.B. and Fink, J. (1978) Proc. Natl. Acad. Sci. USA 75, 1929.

9. Entian, K.-D. and Zimmermann, F.K. (1980) Molec. Gen. Genet. 177, 345.

10. Rogers, P.L., Phil, D., Lee, K.J. and Tribe, D.E. (1980) Process. Biochem. 15, 7.

11. Whittle, D.J., Kilburn, D.G., Warren, R.A.J. and Miller, Jr. R.C. (1982) Gene 17, 139.

12. Bland, R.R., Chen, H.C., Jewell, W.J., Bellamy, W.D. and Zall, R.R. (1982) Biotech. Letters 4, 323.

13. Switzenbaum, M.S. and Jewell, W.J. (1978) Annual Water Pollution Control Federation Conference, Annaheim, Cal. 42 pp.

14. Shimatake, H. and Rosenberg, M. (1981) Nature 292, 128.

15. Gong, C.S., Chen, L.F., Flickinger, M.C. and Tsao, G.T. (1981) (Ed. A. Fiechter) Adv. Biochem. Engineering 20, 93.

16. Chiang, L.C., Hsiao, H.Y., Ueng, P.P. Chen, L.F. and Tsao G.T. (1981) Biotech. Bioengineering Symp. 11, 263.

17. Detroy, R.W., Cunningham, R.L., Bothast, R.J., Bagby, M.O., and Herman, A. (1982) Biotech. Bioengineering 24, 1105.

BIBLIOGRAPHY

Nitrogen-Fixation Project

Brisson, N. and Verma, D.P.S. (in press) "Soybean Leghemoglobin Gene Family: Normal, Pseudo and Truncated Genes". PNAS.

Hyldig-Nielsen, et al. (1982) "The Primary Structure of Two Leghemoglobin Genes from Soybean." Nucleic Acid Res. 10:688.

Roberts, G.P. and Brill, W.J. (1981) "Genetics and Regulation of Nitrogen Fixation." Ann. Rev. Microbiol. 35:207-235.

Bánfalvi, Z., Sakanyan, V., Koncz, C., Kiss, A., Dusha, I., and Kondorosi, A. (1981) "Location of Nodulation and Nitrogen Fixation Gene on a High Molecular Weight Plasmid of R. meliloti."

Friedman, A.M., Long. S.R., Brown, S.E., Buikema, W.J., and Ausubel, F.M. (in press) "Construction of a Broad Host Range Cosmid Cloning Vector and Its Use in the Genetic Analysis of Rhizobium Mutants."

Ruvkun, G.B. and Ausubel, F.M. (Jan. 1981) "A General Method for Site-directed Mutagenesis in Prokaryotes." Nature 289:85-88.

Beringer, J.E., Beynon, J.L., Buchanan-Wollaston, A.V., Johnston, A.W.B. (Dec. 1978) "Transfer of the Drug-resistance transposon Tn5 to Rhizobium." Nature 276:633-634.

Zurkowski, W. and Lorkewicz, Z. (1979) "Plasmid-mediated control of nodulation in Rhizobium trifolii," Arch. Microbiol. 123:195-201.

Nuti, M.P., Lepidi, A.A., Prakash, R.K., Schilperoort, R.A., and Cannon, F.C. (1979) "Evidence for nitrogen fixation (nif) genes on indigenous Rhizobium plasmids," Nature 282:533-535.

Krol, A.J.M., Hontelez, J.G.J., van den Bos, R.C. and van Kammen, A. (1980) "Expression of large plasmids in the endosymbiotic form of Rhizobium leguminosarum, Nucleic Acids Res. 8:4337-4347.

Johnston, A.W.B. and Beringer, J.E. (1975) "Identification of the Rhizobium strains in pen root nodules using genetic markers," Microbiol. 87:343-350.

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HYDROCARBON MICROBIOLOGY WITH SPECIAL REFERENCE TO TERTIARY OIL RECOVERY FROM PETROLEUM WELLS*

prepared by

Ananda Chakrabarty**

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** Professor; Department of Microbiology and Immunology, University of Illinois at the Medical Centre, 835 S. Wolcott Street, Chicago, Ill. 60680, USA.

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A. BACKGROUND AND JUSTIFICATION

Microorganisms are well-known for their ability to attack both aliphatic and aromatic hydrocarbons present in crude oils (1,2). Pure or mixec bacterial cultures may bring about extensive changes in the composition of crude oil by total or partial digestion of hydrocarbon components by complete or co-oxidative metabolism (3). Such microbial growth usually leads to a change in the pH of the medium and an alteration in the physico-chemical properties of the oil.

It is widely recognized that the current technology of oil recovery allows recovery of 30 to 40 per cent of oil from most wells. The bulk of the oil is left behind embedded in the rocks because of its high viscosity. According to 1976 information, in the United States of America a total of 27×10^{10} barrels are known to exist, 6×10^{10} of which have been extracted by primary recovery methods, 3×10^{10} barrels are being recovered by enhanced recovery processes, and the remaining 18×10^{10} barrels remain underground as dead oil.

The usefulness of microorganisms in the recovery of secondary oil cannot be overemphasized. In the United States of America until the mid-seventies, the price of oil has been regulated and maintained at an appreciably lower rate than in most other parts of the world. There was no economic incentive to obtain more oil than could be extracted readily by the available technical means. Since then, there has been a continuing shortage of oil worldwide, resulting in a drastic price increase. Because of the shortage of oil, the price of secondary oil has recently been deregulated and the necessity for increased production of crude oil has become apparent.

Although the microbiological means of recovery of secondary oil has not been taken seriously in the United States of America, some of the concepts of the technology certainly evolved in this country between the forties and sixties (4,56). In contrast, microbial recovery of secondary oil has been subject to field trials in East European countries, particularly in the Union of Soviet Socialist Republics (USSR), Hungary, Poland and Czechoslovakia. Thus, Soviet workers (7) have reported

successful release of oil from reservoirs containing highly viscous oil with a permeability of 1000 md. by injecting mixed cultures of bacterial strains. Extensive field trials in Hungary have demonstrated the applicability of microbial recovery of crude oil (8). In a typical trial, 20 cubic metres of a mixture consisting of 120 m^3 of well water, 40 tons of molasses, 120 kg KNO_3 and 100 kg of sugar was injected into a well along with 100 litres of a mixed bacterial culture comprised of Pseudomonas, Clostridium and Desulfovibrio (9). The well was closed for three months, at the end of which the following results were obtained (Table 1).

Table 1

Changes in reservoir composition after injection of microorganisms in an oil well

Parameter measured	Conditions	
	in original well	after 3 months incubation
Oil production	unspecified	60 per cent higher than original
Oil viscosity	42 centistokes	18 cSt at 40°C
CO_2 gas	0	40 m^3/day
Dissolved CO_2	0	370 mg/litre
pH of reservoir water	9	6

Similar reduction in the viscosity of oil as well as the pH of the reservoir water was noted in other field trials. In another typical field experiment, Karaskiewicz (10) has reported changes of viscosity from 10.67 centistokes to 4.37 cSt with a simultaneous drop in specific gravity from 0.8516 to 0.8320. Microorganisms capable of digesting the heavy fractions of the crude oil producing light fractions and gases are believed to be responsible for such reduction of viscosity (11).

Extensive field trials in Poland have been reported by Karaskiewicz (10). In a typical trial, a well in the Carpathian region produced 4460 kg oil, 16610 kg water and 3780 m^3 of gas per month. The porosity of the

reservoir rock was 13 per cent and permeability of 120 md. The well was injected with mixed bacterial cultures and nutrients, and sealed for three months. At the end of this period, oil production was increased by 360 per cent and the increased production (260 per cent on the average) persisted for six months before declining to the previous level. The pH of the reservoir water fell from 8.7 to 6.7 during this period.

Field trials for microbial recovery of oil in the United States have been few (6) but one report (12) points out that an average increase in oil production in excess of 350 per cent may be expected at a cost varying from approximately 15 ¢ to 50 ¢ per additional barrel of oil. In addition, recent investigations by Petrogen Inc. (personal communication from Dr. A.G. Swan, Research Director of Petrogen) during a recent 100 day test around the end of 1981 on four low-producing oil wells treated with their specially-adapted microorganisms (Biopackage I) demonstrated recovery of oil worth about $24,800. Immediately previous to the treatment with Biopackage I, the four oil wells produced the amount of crude worth about $4,132 during the 100 day test period. Thus microbial treatment of the oil wells has been reported to result in a six fold increase in oil recovery in this particular instance.

Characteristics of Microorganisms

The field trial experiments appear to demonstrate the practical feasibility of microbial recovery of secondary oil from abandoned oil wells. It should be emphasized that the increased production of oil due to injection of microbial cultures has presumably been minimal, since no special precaution was taken to use microorganisms especially designed for this purpose. It appears that in almost all cases, the increased production of oil was basically due to two reasons:

(a) production of large amounts of CO_2 due to bacterial metabolism in the reservoir. Since CO_2 is partially miscible with oil, to swell and become less viscous; and

(b) production of large amounts of acetic, propionic and other organic acids which react with reservoir alkaline carbonate in the formation, leading to increased porosity of the rocks as well as production of more CO_2. Excess of CO_2 and other gases also exert pressure to drive the oil out of the pockets of sand and rock.

The reported success of the microbial recovery of secondary oil, though sporadic, and the continuing increase in the price of oil (presently at about $34 per barrel) have aroused considerable interest in exploring this technology as a viable means of increased oil production, particularly since the secondary oil so produced would be deregulated and draw a premium price. In a Symposium organized by the United States Department of Energy (13), the consensus was reached that microbial recovery of oil is a technically achievable goal and the process can perhaps be considerably enhanced if the microorganisms would have some desirable characteristics. The following characteristics have been deemed particularly desirable:

(a) Production of acids

In addition to producing CO_2 from metabolism, microorganisms should also be able to produce acids in situ. Such acids may then help in the dissolution of carbonate rock structures, increasing the porosity of the rocks to facilitate oil migration and also allowing release of additional CO_2 to push the oil from the embedded rocks. The acids should be strong mineral acids such as HCl or H_2SO_4 or organic acids such as acetic, propionic, succinic, etc.

(b) Production of biopolymers or surfactants

Biopolymers and surfactants are routinely used in flood water to decrease the surface tension of the oil water interface, resulting in decreased viscosity and an easier flow of oil. Ideally, they should be produced in situ by microorganisms, ensuring a continuous supply of such materials in difficult to reach areas of oil-embedded rocks. This also eliminates the problem of transportation, storage and delivery of such materials during water flooding.

(c) Consumption of highly viscous components of the oil

The decreased mobility of secondary oil is primarily due to the high viscosity of the oil. If microorganisms can selectively remove the waxy and other highly viscous components of the oil, resulting in a decrease of the viscosity, then such oil may be recovered easily by water flooding and pumping. Additionally, any loss of sulphur-containing components at this time will result in the recovery of low-sulphur crudes, which usually draw a premium price.

(d) Growth and tolerance

Microorganisms that are deemed appropriate for use in secondary oil recovery must have the ability to grow at a wide range of temperature (35°C to 45°C or beyond), have simple nutritional requirements, preferably with the capability of using cheap substrates (and/or highly viscous components of the oil itself) as sole sources of carbon and using nitrate or fixing N_2 for its source of cell nitrogen. They should be non-pathogenic, ecologically compatible, easy to produce in the laboratory in large quantities and easy to monitor by incorporation of a visible genetic marker of some unique growth-related property.

Advantages and Disadvantages of the Microbial Oil Recovery System:

(a) The major advantage of a microbial system is that it is cheap and simple. Injection of a few hundred gallons of mixed culture and nutrient mixture in the well is relatively simple and straightforward;

(b) The technology for recovery of oil is well-known and the processing does not involve any major technical innovation;

(c) The process may be long-term as bacteria continually digest highly viscous oil fractions releasing acids and light fractions. The acids may continually react with rocks producing more CO_2 and increasing its porosity, thereby releasing more oil

The major disadvantage of the process is the extreme environment in most oil wells that is not conducive to sustained microbial growth. The following points are particularly important:

(a) Temperature: Most oil wells that have been highly productive are quite deep and the temperature may vary from 70 to 95°C in general. Thus, mesophilic and moderately thermophilic microorganisms would not be able to proliferate under such conditions.

(b) Pressure: Although pressure is normally not a factor, higher pressure at some oil wells (50 atm) may reduce biological activity in an otherwise unfavourable environment.

(c) Nature of sands and rocks: Although carbonate rocks can be gradually corroded by the microbially produced acids, the silicate rocks are quite resistant under such conditions.

It would therefore seem that microbial recovery of oil, at least initially, must be confined to shallow wells having predominantly alkaline carbonate rock structures. If the process allows enhanced oil production, thermophilic bacteria having desirable characteristics may be designed based on the characteristics of individual oil wells. Given the vast amount of oil trapped within the rocks, the shortage and spiralling price of crude oil, and the inexpensive nature of the microbial process, it is imperative that microbial oil recovery process should be attempted in a number of oil wells to test the effectiveness of the process.

The major objective of the programme is to enhance the recovery of oil from wells already drilled and from which the bulk of the oil has been pumped out. This programme therefore falls under the category of the secondary and tertiary recovery of oil. Given the fact that there are innumerable oil wells in various developing countries that produce less than one barrel per day, an increase in oil output by microbial technology would help alleviate some of the energy problems in these countries. Another objective is to facilitate the storage and transportation

of crude oil by developing genetically-engineered bacteria that should be able to utilize the waxy components of crude oil, thereby greatly reducing its viscosity. Such low-viscous free-flowing oil not only attracts a premium price, but is less likely to form solid mass during transportation in winter months, thereby greatly increasing the cost of pumping. Free-flowing oil is also much easier to store in underground caverns than the highly viscous oil. Still another objective is to develop microorganisms that would be able to utilize the common sulphur-containing compounds present in crude oil. Desulphurization of crude oil can therefore be accomplished through use of such microorganisms. Along with sulphur-containing compounds organics, many crude oil varieties also contain trace quantitites of metals such as vanadium, nickel, molybdanum, etc. that lower the quality of the oil. Microorganisms capable of selectively removing these metals will also be developed. Lastly, oil spill, either through deliberate washing off of residual oil from oil tankers of accidental due to collision or grounding of oil tankers, can be managed through application of genetically-engineered microorganisms or through use of bacterially-produced emulsifying agents. Such emulsifying agents may also find wide application in cleaning up of residual oil and oily wastes present in drums, tank cars or trucks and oil tankers. Field tests for such micro-organisms or emulsifying agents would be conducted with oil samples taken from oil wells of various developing countries.

B. ACTIVITIES

Since the Oil Recovery Programme would address several other features in addition to tertiary recovery of oil i.e., dewaxing and desulphurization of oil, oil spill clean up and removal of metals, it is clear that several different kinds of activities would be associated with the programme. The major component would be the research and development component, where search in all the above areas would be conducted under a group leader associated with the ICGEB. About three group leaders, each with one post doctoral fellow and one technician will be working in all the five projects. It is anticipated that five trainees from developing countries, preferably from oil producing countries, would be trained in all the above research

areas i.e., one in each of dewaxing, desulphurization and metal ion removal and two in the development of aerobic and anaerobic cultures capable of releasing oil from abandoned oil wells. For countries rich in shale oil or tar sands (such as Canada), some research activities in this area may also be initiated. Yearly workshops will be conducted by this group, in collaboration with petroleum microbiology groups of other institutions in developing and developed countries, on topics such as the role of microorganisms in the degradation of hydrocarbons present in crude and spilled oil, limiting factors (i.e., nature of oil, pH, temperature, O_2 tension, etc.) in such biodegradation, methods for stimulating natural biodegradation, genetic improvements in the biodegradation of hydrocarbons of various chain lengths, etc. In addition, the group would be guided by an advisory committee composed of geneticists, microbiologists, ecologists, geologists and reservoir engineers to advise on the applications of any constructed microorganisms in the open environment, either for oil spill clean up, dewaxing and desulphurization, or in the use of such microorganisms in oil wells for enhanced release of oil.

Extensive pilot plant facilities for successful field trials with the microorganisms will be needed at the ICGEB. Hopefully, there will be centralized facilities for this; otherwise, several fermenter with capacities of 10 to 1000 gallons will be needed. One or more technicians, knowledgable in running fermenters and harvesting cells, should be recruited for this purpose. In absence of a centralized facility, additional 2000 sq. ft. area will be needed to house the fermentation facilities. The cost for the fermenters would be about $100,000.

For actual field trials, the fermenter grown microorganisms along with the nutrients must be transported near the oil well site and the microorganisms, along with the nutrients, need to be pumped in the oil well. A couple of trucks fitted with pumping gear would be needed for this operation. Hopefully, this would be provided by the participating oil well operators or owners.

The major output of the programme would be the construction of genetically engineered strains capable of dewaxing and desulphurizing crude oil, capable of utilizing spilled oil rapidly, capable of removing certain undesirable metals from such oil and finally capable of releasing oil from rock-embedded high-viscosity oil that cannot normally be pumped out by the present day technology. Some of these goals may not be met within the five-year lifetime of the programme. Even if the genetically engineered strains can be constructed, the effectiveness of such strains in an open environment remains to be tested. Recently, another genetically engineered strain capable of digesting a known persistent toxic chemical such as 2,4,5-T has been demonstrated to be quite efficient in removing this pollutant from the environment (14). In this sense, the constructed strains for oil release or dewaxing/desulphurization of crude oil are expected to be effective in their respective areas of application.

Construction of Strains for Tertiary Oil Recovery

Many of the parameters of the microbial enhanced oil recovery have recently been evaluated by a series of studies sponsored by the United States Department of Energy and a major conclusion that has emerged is that about 30 to 40 per cent of the oil wells in Oklahoma, Texas, Louisiana, Kansas, etc. are suitable for microbial enhanced oil recovery processes if the proper microbes and processes can be developed (see report on the United States DOE contract No. DE-AC10-80BC10169, 1981). Assuming that such microorganisms can be developed by the ICGEB and that such microorganisms would be able to allow recovery of 10 to 20 per cent of the residual oil, the tremendous impact of such a technology in the availability of crude oil throughout the world can easily be envisaged.

There would be two fundamental research approaches that the ICGEB should take in constructing appropriate microorganisms. One would be the route taken by the United States Department of Energy, which is to isolate thermophilic, salt-tolerant, anaerobic cultures either from sewage treatment plants or from oil well reservoir cores. Mutational or other genetic improvements can then be attempted to improve the performance of the cultures with respect to their tolerance to extreme pH, salinity,

pressure, temperature, etc., and to improve their ability to convert waxy materials to liquid oil (see reports on United States DOE contracts DE-AT19-78BC30201, DE-AS19-80BC10302, DE-AS19-81BC10507, DE-AC19-80BCk0300, etc.).

It should be emphasized that anaerobes are not particularly suitable for optimum oil release programmes. Anaerobes are not known to biodegrade hydrocarbons at an appreciate rate, so that easily assimilable carbon sources must be supplied in the oil well for their proliferation. Secondly, genetic studies with anaerobes are at their infancy, so that no appreciable genetic improvement can realistically be made at this time. Nevertheless, recent experiments with anaerobic cultures in several oil wells in Texas and Oklahoma by a firm named Petrogen Inc. have demonstrated that out of 24 oil wells injected in six different formations at depths ranging from 300 to 4,600 feet by specially acclimated anaerobic cultures, four showed doubling of pre-injection production levels over a six month period while another 12 demonstrated increases of 50 per cent over pre-injection production levels for a three month period (Dr. A.G. Swan, Research Director, Petrogen Inc., Personal Communications).

The second route to enhanced microbial oil recovery programme to be undertaken by the ICGEB would be to genetically manipulate aerobic cultures, particularly Pseudomonas type, for this purpose. Bacteria belonging to the genus Pseudomonas are well-known for their ability to utilize hydrocarbons, including the C20 and other waxy types. They grow rapidly at temperatures up to 44°C, are tolerant to high pressure, fair amounts of salinity (marine Pseudomonas sp. are tolerant up to high salinity), pH variations, etc. Pseudomonas species are also known to produce polysaccharide biopolymers and can release large amounts of strong acids such as HCl from chlorinated compounds. Genetic tools for improving Pseudomonas species are fairly well advanced. The only drawback with the use of Pseudomonas species is the fact that such bacteria are aerobic, although some species can grow quite well under anaerobic conditions with nitrate as an electron acceptor. In addition to the use of nitrate, it is possible to use the Recombinant DNA techniques to introduce foreign genes into Pseudomonas species that will allow them to produce oxygen in situ in the well. Thus the construction of a Pseudomonas bacterium capable of digesting highly-

viscous waxy material from the oil well, producing polysaccharide polymers and HCl from chlorinated compounds and thriving in the anaerobic environment with in situ production of oxygen can be accomplished at the ICGEB. The construction of such specialized strains and a study of their effectiveness in enhanced recovery of oil from abandoned oil wells will therefore be a major part of the overall programme projects at the ICGEB. Forcing air into the oil wells and allowing genetically manipulated Pseudomonas strains to thrive for oil release will also be a part of the ICGEB field trials.

Dewaxing and Desulphurization of Crude Oil

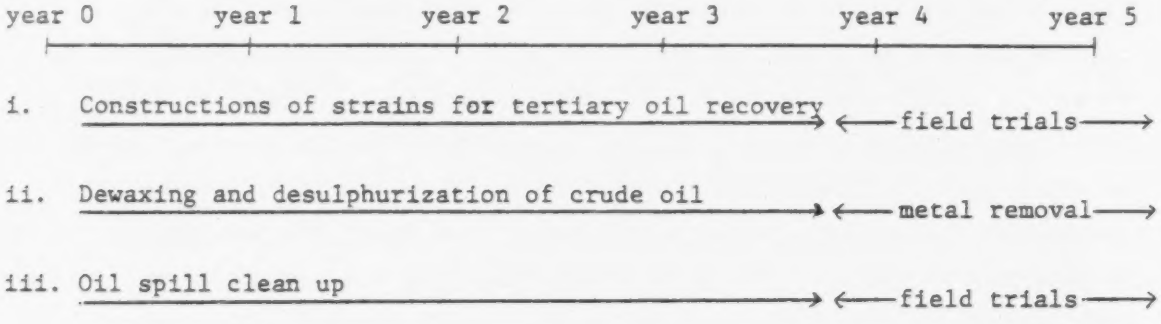
Recent genetic studies with Pseudomonas species have demonstrated that it is possible to specially breed in the laboratory strains that can utilize a known, persistent compound such as the herbicide 2,4,5-T (15). Since there are no naturally occuring microorganisms that can utilize 2,4,5-T as a sole source of carbon, the laboratory-breeding of such a culture raises hope that similar strains capable of utilizing other recalcitrant compounds such as dibenzothiophenes or furans, known to occur in crude, can be developed. The use of bacterial cultures capable of dissimilating dibenzothiophenes to a water soluble product in removing 90 per cent of the sulphur from crude oil has now been demonstrated. Since the presence of wax or sulphur-compounds in crude leads to transportation and pollution problems, development of cultures capable of dewaxing or desulphurizing the crude would be very useful. In addition, as mentioned in the Enchanced Recovery section, such characteristics imparted in the strains to be used for EOR would not only release the oil from low-producing wells but the oil would demand a premium price because of the dewaxing and desulphurization in situ. Such microorganisms would be particularly useful for crudes obtained in the People's Republic of China, Indonesia, Nigeria, Venezuela, etc. Removal of some of the metals present in crude oil in certain countries will also be attempted through the use of genetically manipulated bacteria.

Oil Spill Clean-Up

Another project that the ICGEB should be interested in is the construction of multi-plasmid strains capable of utilizing spilled oil rapidly. Although the general patent on construction of such strains

and their use in the area of the spill is owned by the General Electric Company, there are numerous other improvements that can be made over such strains. The details of the construction and genetic manipulation of such strains have been described (16). Similar approaches could be taken at the ICGEB to construct a more efficient strain. The usefulness of such a strain in removing spilled oil would be tested over a variety of areas, such as fresh water lakes and rivers, coastal and far-off seas, etc., to determine the effectiveness of the strain. If necessary, separate strains would be constructed, depending upon the nature of the spilled oil, the ecological status of the body of water (i.e., fresh water lakes versus seas), etc.

C. WORK PLAN



D. CO-OPERATION WITH OTHER INSTITUTIONS

Co-operation with petroleum institutes and other relevant institutions will be secured in the implementation of the programme.

Co-operation with national institutes and agencies for carrying out effective field trials will be a significant component of the work programme.

E. PREREQUISITES

A major prerequisite of the programme is the appropriate academic qualifications of the trainees from developing nations. Such trainees must have very good background in microbial physiology and genetics with considerable knowledge in analytical techniques. Preliminary screening for trainees should also take into account the type of institution the trainees will go back to, that is, if the trainee is employed in a petroleum research institute rather than a medical institute or a university department of life sciences, then the possibility of the trainee using his skills to pursue research in microbial recovery of oil or clean-up of oily waters is much higher in the petroleum research institute than in the other two. The same rationale applies to trainees from oil producing countries than from countries without any oil, in as much as trainees from the former countries will have ample opportunity to practice their skills than if the trainees were recruited from the latter.

The cost of the field trials could be shared between the ICGEB and the oil well operators, particularly if the developed microorganisms are found to be effective in enhancing recovery of oil by several folds. Since growth of microorganisms or supply of nutrients are not expensive items, the cost for each injection in the oil well should not exceed $5000. In some cases, this cost may be recouped by the earnings from the recovered oil. In general, the total cost of field trials per year should not exceed $100,000.

F. FINANCIAL REQUIREMENTS

The following staff will be required for implementing this work programme:

- two senior scientists
- four junior scientists
- three post doctoral fellows
- four technicians

15 trainees are expected to participate in the development of the programme.

Five-Year Budget

STAFF		(US$ thousands)
(first year 40 per cent, second year 60 per cent of full operation)		
Senior scientist	8 man year	600
Junior Scientist	16 man year	720
Post doctoral scientist	12 man year	288
Technicians	16 man year	272
Subtotal		1,880
Management of the Centre and Supporting Personnel		489
Total Staff		2,369

OPERATIONAL ACTIVITIES		
Visiting Scientists	20 man months	160
Expert group meetings	2 man months	50
Advisory services	15 man months	150
Training	30 man year	675
Information material		15
Purchase of chemicals etc.	56 man unit year	560
Associateship		75
Miscellaneous		72
Total Operational Activities		1,757
Total Work Programme		4,126

ANNEX I

EQUIPMENT REQUIREMENTS

As mentioned previously, this programme would involve about two to four ICGEB staff and five to six trainees from developing countries. This staff does not include the personnel necessary for conducting the field trials. Since the field trials will be conducted in collaboration with oil well operators and their engineering personnel, the need for having such people at the ICGEB is not obvious.

The space requirement for this programme would be about 5,000 sq. ft. of a laboratory with working benches, chemical hoods and biological containment systems. This does not include the office spaces for the personnel. It would be advisable to separate the genetic manipulation part of the laboratory from the microbial physiology laboratory to avoid excessive contamination problems. The laboratory should be equipped wigh modern preparative and analytical equipments such as:

- ultracentrifuges (Beckman L8 or DuPont OTD65)
- recording spectrophotometers (i.e. Gilford 2600 or Beckman 35)
- RC-2B and RC-5 centrifuges
- gas chromatograph (Varian 3700)
- high pressure liquid chromatography (Perkin Elmer series 2)
- liquid scintillation spectrometers (Packard Tri Carb or Beckman L57000)
- Gilson Oxygraphs and differential respirometers
- electrophoresis and TLC densitometers
- lyphilizers
- French Pressure cells and sonicators
- shakers and incubators

In addition, the research group must have access to electron microscopes, combined gas chromatography - mass spectrometry, IR, NMR and ESR analytical equipments. The cost of such equipments could go as much as $200,000.

REFERENCES

1. Gutnick, D.L. and Rosenberg, E. 1977. Ann. Rev. Microbiol. 31, 379-396.
2. Abbott, B.J. and Gledhill, W.E. 1971. Adv. Appl. Microbiol. 14, 249-388.
3. Perry, J.J. 1979. Microbiol. Rev. 43, 59-72.
4. ZoBell, C.E. 1946. U.S. Patent 2,413,278.
5. Bond, D.C. 1961. U.S. Patent 2,975,835.
6. Frick, J.R. 1972. J. Petrol Technol. 24, 1469-1470.
7. Senjukov, B.M., Yulbarson, V.M., Talbukina, H.H. and Sisenina, E.P. 1970. Mikrobiologia 39, 705-710.
8. Jaranyi, I., Kiss, L., Szalanczy, G. and Szolnoki, J. 1965. Vorträge III. Inter. Wiss. Konferenz Geochem. Microbiol. 2, 633-650.
9. Jaranyi, I., Kiss., L. and Szalnczy, G. 1967. Magyar All. Foldtari Int. Jelentese 1967, 345-349.
10. Karaskiewicz, J. 1973. Nafta I, 144-151.
11. Karaskiewica, J. 1968. Biuletyn Instytutu Naftonwege 1, 1-3.
12. Johnson, A.C. 1979. in Conference on Microbiological Processes Useful in Enhanced Oil Recovery (US DOE Conf. 790871), pp 30-34.
13. Conference on Microbiological Processes Useful in Enhanced Oil Recovery. 1979. U.S. Department of Energy, Conf. 79871, UC-92).
14. Chatterjee, D.K., Kilbane, J.J. and Chakrabarty, A.M. 1982. Appl. Environ. Microbiol. (in press, August issue).
15. Kellogg, S.T., Chatterjee, D.K. and Chakrabarty, A.M. 1981. Science 214, 1133-1135.
16. Chakrabarty, A.M. and Brown, J.F. 1978. in Genetic Engineering (Chakrabarty, A.M., ed.). p. 185-195. CRC Press, West Palm Beach, Florida.